EXPERIMENTAL AND ANALYTICAL STUDY OF CARDIOVASCULAR CONTROL LOOPS

By N.A. Normann, M.D.

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FOREWORD

This research was conducted at the Hamilton Standard Division of United Aircraft Corporation under Contract NASW 1350 with the Biotechnology and Human Research Division of the Office of Advanced Research and Technology, the National Aeronautics and Space Administration. The contracting officer was Herbert S. Snyder.

The principal investigator was Dr. Nils A. Normann and collaborating in the Program were the Misses Mary Ann Bianchi and Analee Gelman.

Animal care and experimental procedures were executed in accordance with the "Guiding Principles in the Care and Use of Animals" put forth by the American Physiological Society. A licensed veterinarian was responsible for animal care procedures.

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By N. A. Normann, M. D.
Biomedical Systems Department
Hamilton Standard
A Division of United Aircraft Corporation
Farmington, Connecticut

SUMMARY

The purpose of this program has been to gain access to the information flowing in intact autonomic nerves, specifically as it relates to circulatory control. To this end, methods have been developed with which neural data have been obtained from representative efferent and afferent visceral nerves in the cat. Briefly summarized, the results are the following:

Technological

I. Electrodes have been developed which:

   a. Are well tolerated and cause a minimum of trauma.

   b. May be placed on small and relatively inaccessible nerves.

   c. Minimize bio-electric interference (ECG, EMG).

   d. Are suitable for implantation.

II. Methods have been developed for implanting electrodes and for monitoring nerve traffic in the intact animal (hard-wire system).

III. Cross-correlation technique has been the principal method of nerve impulse identification. Signal conditioning and processing were sought and optimized for selectivity with respect to the various classes of nerve impulses.

IV. Signal averaging was applied to the cross-correlated data, thus improving the accuracy of the nerve patterns occurring during cardiac and/or respiratory cycles.

Experimental

I. Probably the most significant findings obtained in this study pertain to the functional organization of sympathetic outflows and to sympathetic responses to hypoxia:

a. Under acute experimental conditions, in response to hypoxia, the sympathetic outflow patterns in upper and lower thoracic segments may be entirely different. Thus, the outflow in the third segment may display only a decline, while at the same time the outflow in the greater splanchnic nerve may increase several fold.

b. The sympathetic responses to hypoxia appear dependent upon the pre-hypoxic level of activity; from a previously high level (if not caused by CO_2) it is more likely to show a decrease only.

c. Elevated concentrations of CO_2 (up to 10 percent) to varying degrees increased sympathetic outflow before and during hypoxia; post-hypoxic "depression" was shortened. Tendency to cardiac and/or respiratory modulation was accentuated; combination of hypoxia and hypercarbia might cause respiratory "bursts" to appear at double the fundamental frequency (Figure 5.).

d. In the greater splanchnic nerve, the outflow pattern in B-fibers may differ both quantitatively and temporally from that observed (simultaneously) in efferent C-fibers.

e. "Steady state" sympathetic outflows vary from animal to animal in a seemingly unpredictable manner.

II. Attempts to correlate adrenal medullary secretion of catecholamines with greater splanchnic nerve traffic were unsuccessful. The data suggest that in response to hypoxia, the animal secreting catecholes with a higher norepinepherine - epinephrine ratio tends to maintain a higher arterial blood pressure.

III. Baroreceptor impulses from the aortic arch were monitored in the intact left vagus. Experiments with ganglionic block indicate that sympathetic "tone" may reduce the number of baroreceptor impulses elicited by a given blood pressure and pulse wave.

IV. Simultaneous recordings of baroreceptor impulses from the aortic arch and from the carotid sinus demonstrated that if they are added algebraically the resulting waveform (envelope) represents both spatial and temporal summation. On the basis of such a recording, the pulse wave velocity in the common carotid artery (cat) was calculated to be 16.7 meters per second.

V. Afferent impulses originating in pulmonary stretch receptors were obtained from the intact right vagus nerve in the neck. The pattern was found to parallel closely the radial (combined radii) expansion of the chest.

VI. Attempts to analytically isolate efferent or slow conducting afferent fiber groups in the intact cervical vagus were unsuccessful; at the present stage the methods lack the necessary sensitivity.

VII. Experiments with implanted electrodes on autonomic nerves demonstrated that this is feasible. However, to obtain satisfactory results improvements are needed with respect to electrode structure and to noise interference.

VIII. During the study it became evident that autonomic nerve data should be obtained in the intact animal; anesthesia and surgery profoundly distort "normal" autonomic circulatory control modes.

IX. Present limitations of the methods have been discused.

BACKGROUND AND OBJECTIVES

Neuro-humoral factors are intimately involved in control of the circulation of blood. The ability of the cardiovascular system to respond appropriately to sudden changes and to increased demands is to a great extent due to the existence of neural control loops - providing system adaptation with suitable speed (time-constants) and stability.

The neural, autonomic* pathways involved and their functional significance are well known in a general, qualitative way. In contrast, knowledge of the quantitative, operational characteristics of the autonomics in the intact organism is rather scant; conclusions in this regard are reached mainly by interpretation of "symptoms", i.e., by inference. This lack of direct, pertinent information pertains both to "normal" and "abnormal" states; knowledge of the exact mechanisms involved in circulatory adaptive processes is fragmentary - whether the adaptation is in the response to a coronary occlusion or to a change in gravitational load. Observations of hemodynamic quantities, such as pressures and flows are by themselves apt to be of limited value, inasmuch as they represent parameters which the system tends to control. Direct access to information flowing in relevant control loops would be expected to produce further insight into system behavior and might also provide a sensitive, quantitative indicator of certain adaptive processes.

In consonance with the considerations briefly outlined in previous paragraph, the overall objective of the program has been to gain access to information flowing in intact autonomic nerves, relevant to circulatory control. The specific objectives have been -

(1) To develop the necessary methodology and

(2) To apply the methods in an analysis of autonomic functional organization.

* "Autonomic" is here used in a wider sense, thus including visceral sensory pathways, e.g. from baroreceptors.

INTRODUCTION

The Program has been supported by two consecutive contracts, NASW 1061 and NASW 1350, and consists therefore of two phases. During the first contract period (Phase I) it became evident that decoding of autonomic nerve traffic entailed more obstacles and pitfalls than anticipated; consequently, the major efforts had to be directed toward methodology. Briefly summarized, the results of the first phase were: (a) Development of basic methods; (b) Demonstration of feasibility; (c) Determination of method limitations; (d) Some initial observation pertaining to autonomic efferent and visceral afferent impulse patterns.

During the second phase, which this report is concerned with, the efforts to improve methods were continued. Experimentally, we have sought to substantiate and extend the findings in Phase I, especially those which pertain to autonomic (sympathetic) functional organization. As experience was gained, it became evident that observations made under acute experimental conditions are of limited value: anesthesia and surgery profoundly alter the character and responsiveness of autonomic outflows. For a meaningful analysis (and if the results are to be extrapolated to man) neural information should be obtained from the intact animal. Considerable time and effort have therefore been expended on electrode implant technology.

GENERAL METHODS

Nerve Traffic Analysis

Raw data obtained from an intact nerve consist of impulses occurring in more or less random fashion. If the nerve is composed of two or more fiber groups, which is frequently the case, it is difficult or impossible to determine with any degree of accuracy the activity in each group by a mere inspection of an oscilloscope trace. Anatomically and functionally, however, the fiber populations may differ considerably in terms of direction and velocity of impulse conduction, the velocity being proportional to fiber diameter. These differences have been utilized as means of identifying the individual nerve impulses. Douglas and Ritchie (reference 1) developed a method based on coalition between an artificial antidromic volley and spontaneous ortho-dromic impulses; the cancellation occurring at a given distance served as identification and as a measure of population activity (reference 1). The method is limited in its applicability because it introduces and relies on artificial stimulation, a feature which frequently necessitates cutting the nerve. Yasargil (reference 2) in a study of naturally occurring efferent and afferent impulses in the intact phrenic nerve, used two pairs of electrodes and displayed the two channels of recorded nerve signals on a dual beam oscilloscope; the polarity of an impulse at the active electrode indicated direction, and the electrode-distance/time ratio gave the speed of conduction (reference 2). The same principle has later been employed more efficiently by the use of electronic analog computers (references 3, 4, 5, 6)and constitutes the basis for the method employed in the present study. Recorded from two sites on a nerve, "A" and "B", two channels of nerve signals are obtained. The time difference for impulses common to the two channels is determined by feeding the signals of one channel through a variable delay line and from the other channel directly. Depending on the travel time between A and B, at a certain delay an impulse recorded at the two sites will appear coincident at the two outputs. The coincidence of signals may be established by use of an AND gate or by multiplying the outputs from the two channels, resulting in a cross-correlation function. The distance between electrodes divided by the matching delay time corresponds to speed of conduction; direction (efferent or afferent) is determined according to which channel is being delayed.

In practice, platinum electrodes were placed under the intact nerve at two separate sites. After suitable amplification (gain: 10,000) the two channels of nerve signals were recorded on tape together with other relevant data, such as blood pressures, ECG, respiration. The first step in the analysis of the neural data was to identify the fiber groups involved: a certain portion of data (30 to 60 seconds in length) was transferred to a tape loop. From the loop the data was fed through the correlator, and for each revolution of the tape loop the delay was automatically increased in 25 msec. steps. On an X-Y plot a curve was obtained indicating at which delays (afferent or efferent) activity was present. For example, if the electrode distance was 12 millimeter and a peak appeared when proximal (central) electrode channel was delayed 2 msec., it would indicate activity in efferent fibers conducting impulses at the speed of 6 meters per second (6 millimeter per msec.).

After such scanning procedure, the correlator was set at a delay corresponding to a given conduction speed (in the above example at 2 msec.), and the nerve data from the original tape recording were then run through the correlator continuously. After suitable integration, the output was recorded on a polygraph simultaneously with other data (e.g. hemodynamic). Thus, composite records were obtained - of the type reproduced in this report.

Signaling Averaging

Cross-correlation, as just described, is inherently a statistical method by which signals may be retrieved out of "noise". The effective signal/noise ratio greatly determines the amount of information which can be obtained.

Under certain circumstances, the flow of impulses in autonomic nerves (e.g. sympathetic efferents) may display a distinct modulation in phase with either cardiac or respiratory cycle or both. The repetitive pattern thus encountered lends itself to further statistical treatment of the data. In this study a "Computer of Average Transients" (CAT 400, Technical Measurement Corporation (TMC)) was employed. After cross-correlation, neural and other data were fed into the CAT, utilizing trigger signals obtained from ECG, arterial pulse, or respiration. During the sample period (sweep) following each trigger impulse, data were stored in the computer's memory. In this manner, by summing the data from 20 to 40 samples, the resulting signal definition was significantly improved.

Catecholamine Determinations

In the experiments performed during the first phase of the program, involving adrenal medullary output, total catecholamines were determined. Because of the significant differences in their physiological effects, the two catecholamines, epinephrine and norephinephrine, have been determined separately in the present series. The lowest concentration permitting reliable measurement is 0.03 microgram (± 0.005) per milliliter (ml). The size of each sample taken from the adrenal vein was 0.3 ml. The sensitivity of the method has sufficed for most experiments. In some preparations, however, the output of catecholamines has been too small to be measured with out present methods, equipment, and sample size.

PRESENTATION OF EXPERIMENTAL MATERIAL

The experiments performed (a total of 97) deal with several subject matters. For the sake of clarity, this report will deal with each of the following four experiment categories separately:

Sympathetic Outflow During Hypoxia

Adrenal Medullary Catecholamines and Circulatory Responses to Hypoxia

Monitoring of Baroreceptor Signals

Monitoring of Signals from Pulmonary Stretch Receptors

A general discussion of methods used in the various experiments will precede the detail discussion of the individual experiments. Each section covering a specific experiment category will contain, as applicable, information on specific methods, data, results, and conclusions pertinent to that series of experiments.

Methods - General

The animals were anesthetized with chloralose (30 mg/kg) and uretane (250 mg/kg), or pentobarbital sodium (30 mg/kg), depending on the objectives of the experiment. After the introduction of an endotracheal tube, anesthesia was maintained with a mixture of nitrous oxide and oxygen in the ratio of three to one. An air-driven pump, connected to the endotracheal tube, provided respiratory ventilation for the open-chest experiments.

Arterial and venous pressures were obtained through teflon catheters placed in the right common carotid artery or femoral artery and the external jugular vein. The transducers used were Statham P23AA and P23BB. The electrocardiogram was obtained by the use of subcutaneous needle electrodes. Nerve signals were amplified by cascading two Tektronix 122 battery-operated preamplifiers (a total gain of 10,000), with a band width setting of 80 to 10,000 cycles/second. The output from these preamplifiers was fed into an Ampex FR-110 tape recorder. Transducer signals and the amplified ECG were recorded simultaneously, both on tape, and by an Offner polygraph machine (504C).

The nerves studied in the four/experiment categories were dissected free with great care. The epineurium was left intact in order to avoid interference with blood supply and possible damage to the nerve.

Contact with the nerves was made with 2 mil iridium-platinum wire mounted on silastic sheeting (3 mils thick). This wire was soldered to a coiled copper wire (coated

with Silastic 382 elastomer) which was connected to a shielded multi-strand silver wire. In the chronic animals, this extension was threaded subcutaneously and brought to the surface (back of neck) where it was soldered to a miniature connector (Cannon). This was used as a point of contact for recording nerve signals in the unanesthetized animals.

Sympathetic Outflow During Hypoxia

Of the multiple, physiological responses elicited by a reduction in available oxygen, that of the cardiovascular system is of particular interest. The ability to withstand the hypoxic stress is to a great extent determined by cardiac performance and by vascular adjustments. These cardiovascular changes come about by a complex interplay of a variety of "regulatory" factors, such as hemodynamic (hydraulic), auto-regulatory, biochemical, hormonal, and neural. The experiments to be described are concerned with the nerual component in the physiological response to acute, short-term hypoxia, anoxia or asphyxia. More specifically, an effort has been made to elucidate the character of sympathetic outflows under these conditions. It is well known that an "activation" is the overall sympathetic response to acute anoxia, thus representing a major factor in cardiovascular adjustments; the exact nature of this activation, however, is much less known.

Experimental Data. - Sympathetic outflows strongly affect both the pumping performance of the heart and the impedance - capacitance characteristics of the vascular bed. Accordingly, in this series of experiments, the sympathetic nerve data obtained fall into two categories: a) those obtained in cardiac nerves and b) those obtained in nerves supplying the intestinal area (splanchnic nerves).

Data from a representative experiment of the first experimental category, (involving activity in the inferior cardiac nerve), are reproduced in Figures 1 through 4. In Figure 1 is depicted the response to graded hypoxia (bottom half is the continuation of upper half). The neural output was processed with two time-constants of integration in order to visualize detail (second trace from top), and the overall envelope (third trace from top). The burst-type activity was caused by two types of modulation, cardiac and respiratory — both increasing in amplitude with deepening hypoxia. It is noteworthy that the burst pattern prevailed even at five percent oxygen (O_2); during the final 100 percent nitrogen (N_2) the firing became continuous. During recovery (100 percent O_2) the nerve activity went down to near zero (= one major division); concomitantly the blood pressure (BP) rose and displayed the usual overshoot. It may be surmised that the onset of bradycardia during the anoxic period was related to the dropoff of sympathetic outflow.

Details of the modulation synchronous with the cardiac and respiratory cycles may be seen in Figure 2. Processed with a 5 msec time-constant (second trace from top), the activity displayed a striking burst pattern; between bursts the amplitude is near zero (= one major division).

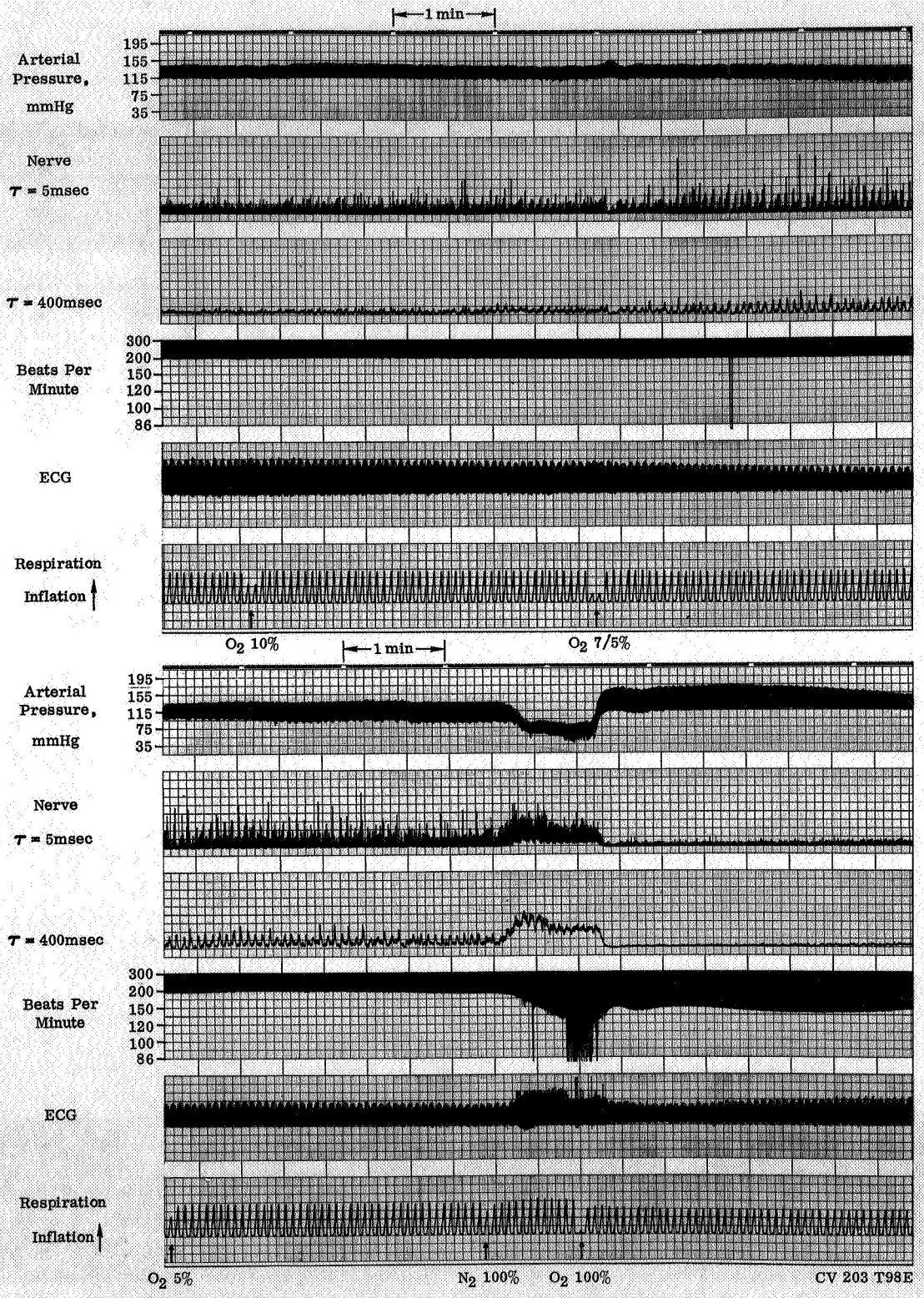


Figure 1. Response to Graded Hypoxia

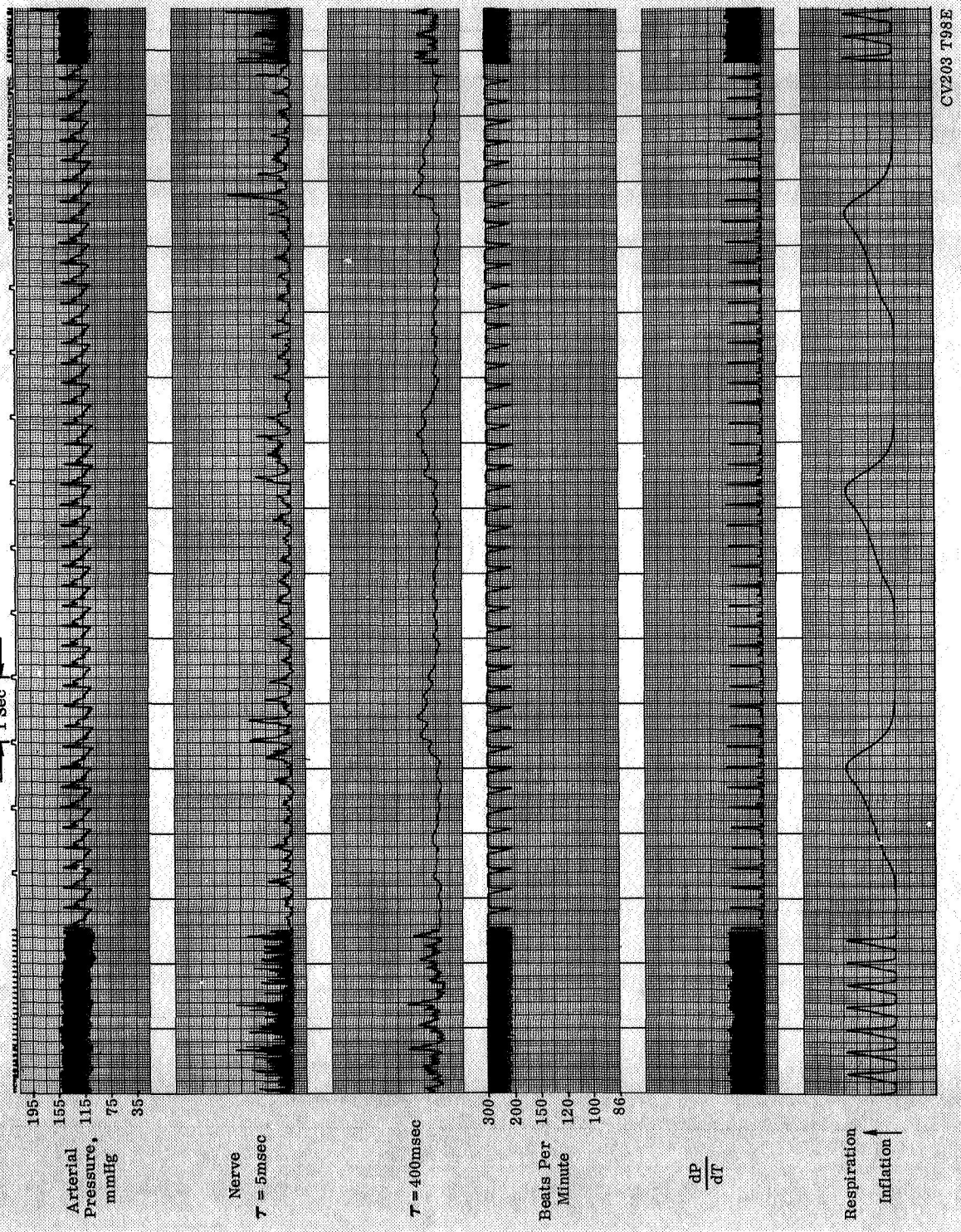


Figure 2. Modulation Synchronous With Cardiac and Respiratory Cycles

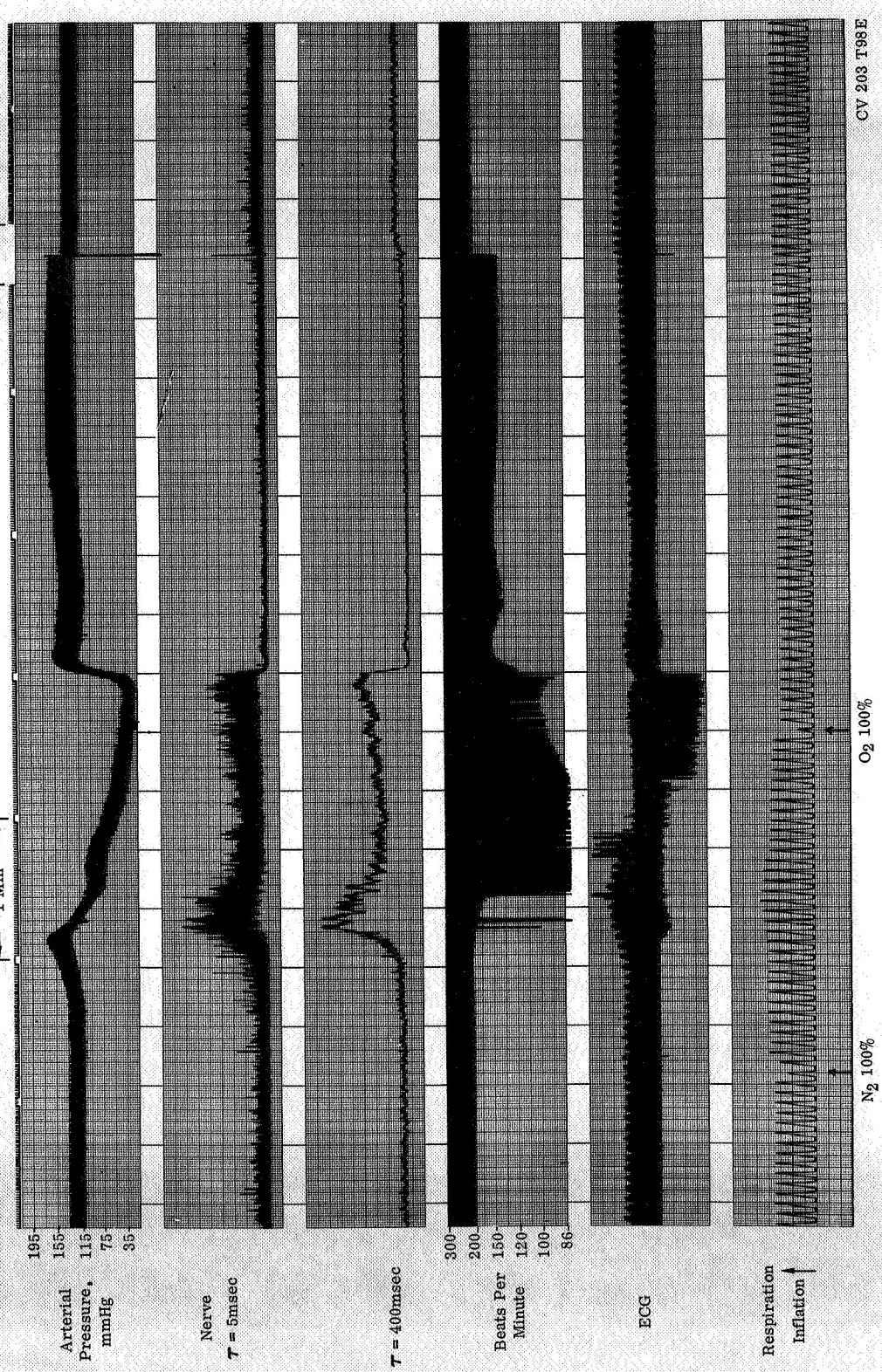


Figure 3. Difference in Magnitude of Sympathetic Response to Acute Respiratory Anoxia When Not Preceded By Graded Hypoxia

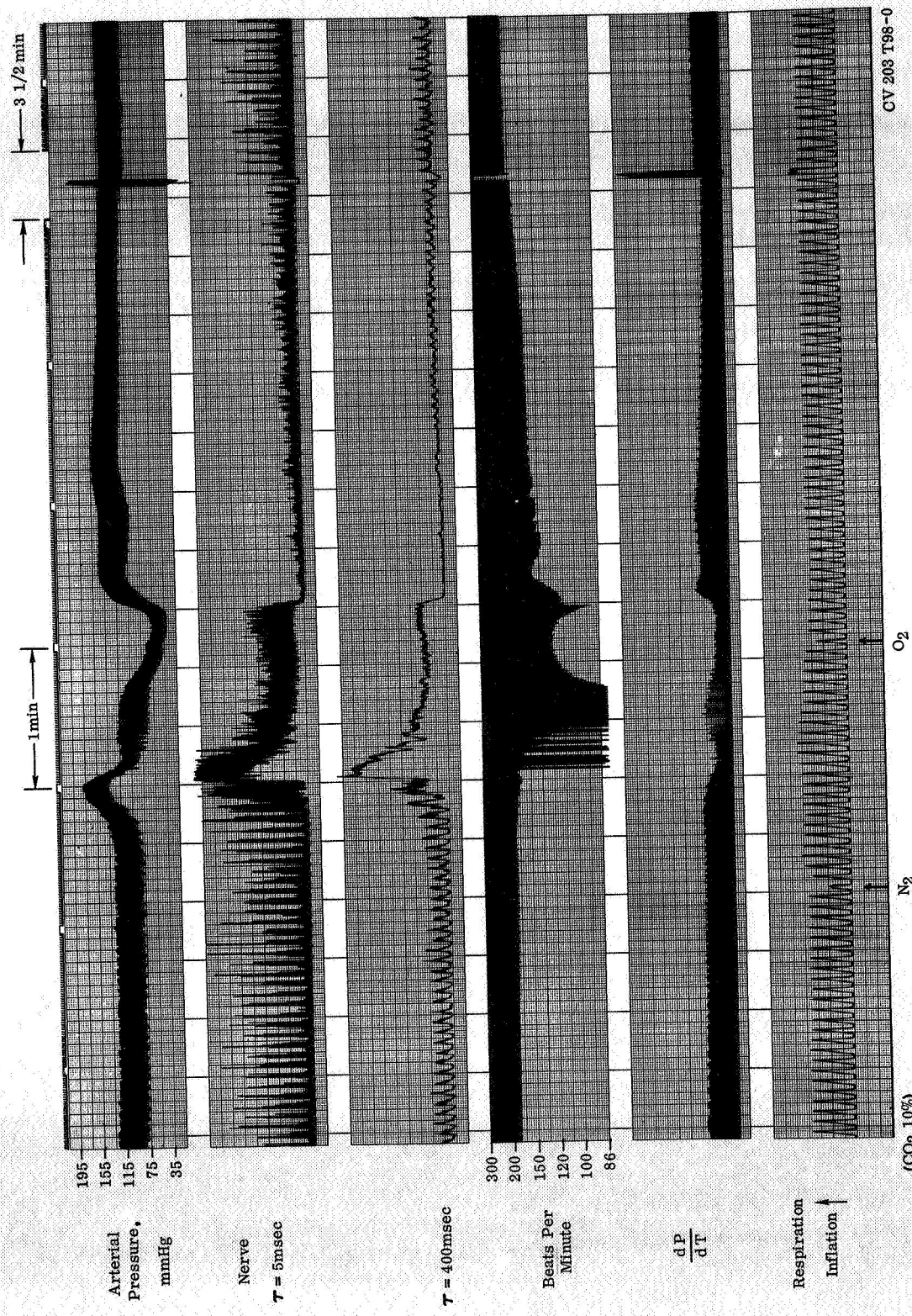


Figure 4. Effects of Carbon Dioxide on Sympathetic Response to Anoxia

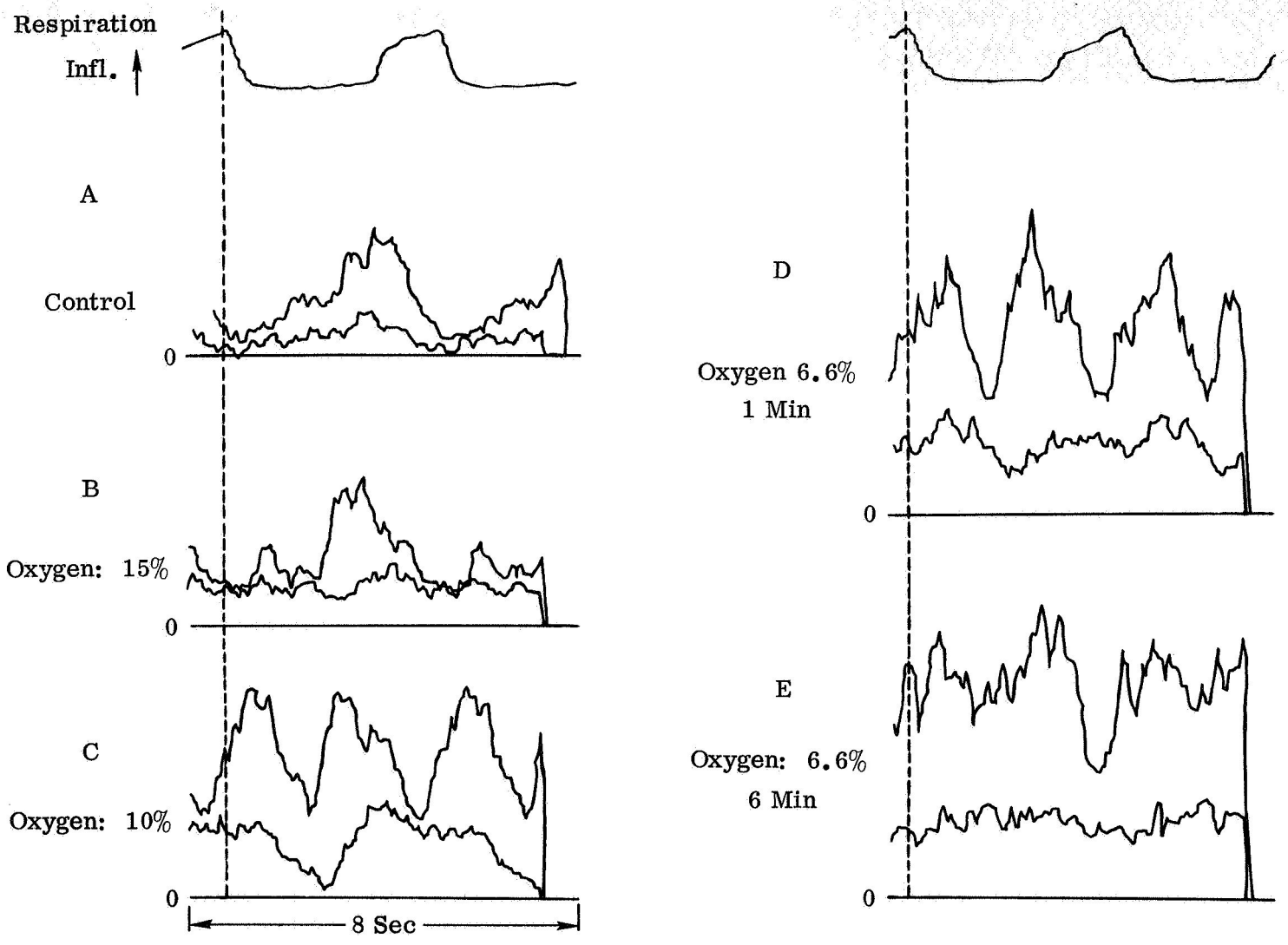
The temporal relationships to the arterial pulse-wave and to the pulmonary phase may be observed: peak amplitudes occur respectively during diastole and during pulmonary deflation. (The curarized animal was ventilated with a respirator.)

In Figure 3 is illustrated the difference in the magnitude of sympathetic response to acute respiratory anoxia — when it was not preceded by graded hypoxia: the neural output was roughly twice as big as that reproduced in Figure 1. (Significantly, the acute anoxia was accompanied by an initial, transient rise in BP.)

The effects of carbon dioxide (CO_2) on the sympathetic response to anoxia was observed in the same preparation - as depicted in Figure 4. With the introduction of ten percent CO_2, the neural bursts became strikingly respiratory and of high amplitude. (The neural trace, 400 msec time-constant, was recorded with 0.4 the grain used in previous recordings.) The magnitude of the sympathetic response indicates that those previously described were not maximum; evidently, in the presence of CO_2 the neural system was capable of higher output in response to anoxia. It is of interest to note that although the initial BP rise reached a level comparable to that seen in Figure 3, the relative change was greater. Again, the data suggest a causative relationship between the reduction of sympathetic outflow and the onset of bradycardia during anoxia.

The augmenting effect of CO_2 may have on the sympathetic response to hypoxia (anoxia) is further illustrated in Figure 5. Data for the figure were obtained in an experiment dealing with the other category of sympathetic outflows: those supplying the peripheral vascular bed.

In this particular experiment, one succeeded in placing one electrode on the ninth thoracic ramus and one distally on the sympathetic trunk. (Usually, the sympathetic rami are too short for electrode placement.) The nerve activity displayed a pronounced respiratory modulation; cardiac cycle modulation was small. In order to obtain more precise information concerning modulation wave-forms and temporal relationships, the cross-correlated nerve data were fed into a CAT 400 (Computer of Average Transients). Trigger was derived from respiration transducer voltages and each tracing (Figure 5) represents the average of 20 samples. In each section the lower and the upper trace depict the hypoxic response in the presence of 1 and 7.5 percent CO_2 respectively. When changing from one O_2 level to another, the sympathetic response comes on quickly and reaches a steady state in the course of a few minutes (-dependent upon a relatively "steady state" of the circulatory response). The tracings in section "B" and "C" were obtained four to five minutes after the new O_2 level was introduced. In the sympathetic response to hypoxia (Figure 5) two features stand out: the high amplitude and the burst pattern occurring in the presence of CO_2 7.5 percent. With respect to the latter, a doubling of burst frequency is in evidence already with O_2 15 percent, becoming pronounced with O_2 10 percent. At low O_2 tensions the neural discharge tended to become continuous. It is noteworthy that during hypoxia the bursts in the presence of one percent CO_2 was lagging those in the presence of 7.5 percent CO_2.



Sympathetic Outflow In Ninth Thoracic Ramus
During Hypoxia With CO_2 1% & 7.5%

CV 115 T64A-B

Figure 5. Augmenting Effect of Carbon Dioxide on Sympathetic Response to Hypoxia (Anoxia)

In the experiments described so far, sympathetic activity was monitored at the level of outflow either to the heart or to the peripheral vascular bed. In the experiment to be described, neural activity was monitored at both levels simultaneously. The responses to a period of anoxia are depicted in Figure 6. The nerve tracings - second, third, and fourth from top represent the following sympathetic outflows: ascending, preganglionic in the sympathetic trunk at the level of third thoracic segment (Th. III); preganglionic in the greater splanchnic nerve; postganglionic in the greater splanchnic nerve. The differences between the respective patterns are rather striking: at the third thoracic segment the pre-anoxic, neural output was relatively high and displayed only a reduction in response to anoxia; the splanchnic efferents, on the other hand, responded with a great increase from a relatively low pre-anoxic level of activity. Furthermore, significant quantitative and temporal differences is seen between preganglionics (7.5 m/sec) and postganglionics (1 m/sec) in the greater splanchnic nerve.

It is of interest to note that the Th. III response pattern in this figure (experiment CV-93) contrasts sharply to that depicted in Figure 3 (experiment CV-203). The opposite types of response observed in two experiments may be related to two important variables, namely anesthesia and circulatory status. In experiment CV-203 (Figure 3) the animal was anesthetized with pentobarbital and was normotensive; in experiment CV-93 (Figure 6) the animal was anesthetized with chloralose-urethane and was hypotensive before anoxia was introduced.

Discussion of Results. - As emphasized in the previous report, in response to anoxia (or asphyxia) the sympathetic outflow in the upper thoracic segments may be entirely different from that in the lower segments. Thus, sympathetic outflow to the heart may differ from that reaching the peripheral vascular bed (e.g. in the intestinal area). In the acute experimental situation, this differentiation is evident before additional stress in the form of anoxia is introduced. Frequently, the activity in upper thoracic segments is relatively greater (when compared to maximum) than in lower segments. Conceivably, the open chest in these cases represents a contributing factor. As for the differentiation in response to hypoxia, the pre-existing level appears important. If the level is high, the only effect of hypoxia may be one of depression. In this context the anesthetic used and the depth of anesthesia contribute relevant variables. While chloralose-urethane anesthesia has the reputation of not depressing sympathetic activity, barbiturates are well known to do so. Typically, in the experiment depicted in Figure 3 (one of the few performed under pentobarbital anesthesia in this series), the sympathetic outflow in the inferior cardiac nerve was relatively small, but it could be increased several fold by anoxia. In the experiment depicted in Figure 6, conducted under chloralose-urethane anesthesia, from a pre-existing high level of activity only anoxic depression was encountered.

The importance of "the past history" is also demonstrated by the difference in response when anoxia is introduced acutely and when it follows a period of hypoxia. The smaller response in the latter instance again indicates that the higher the activity, the more susceptible it is to the depressant effect of anoxia. An important exception is the effect of carbon dioxide (CO_2): in spite of an increased sympathetic outflow; the response to hypoxia and anoxia is not reduced, but rather augmented.

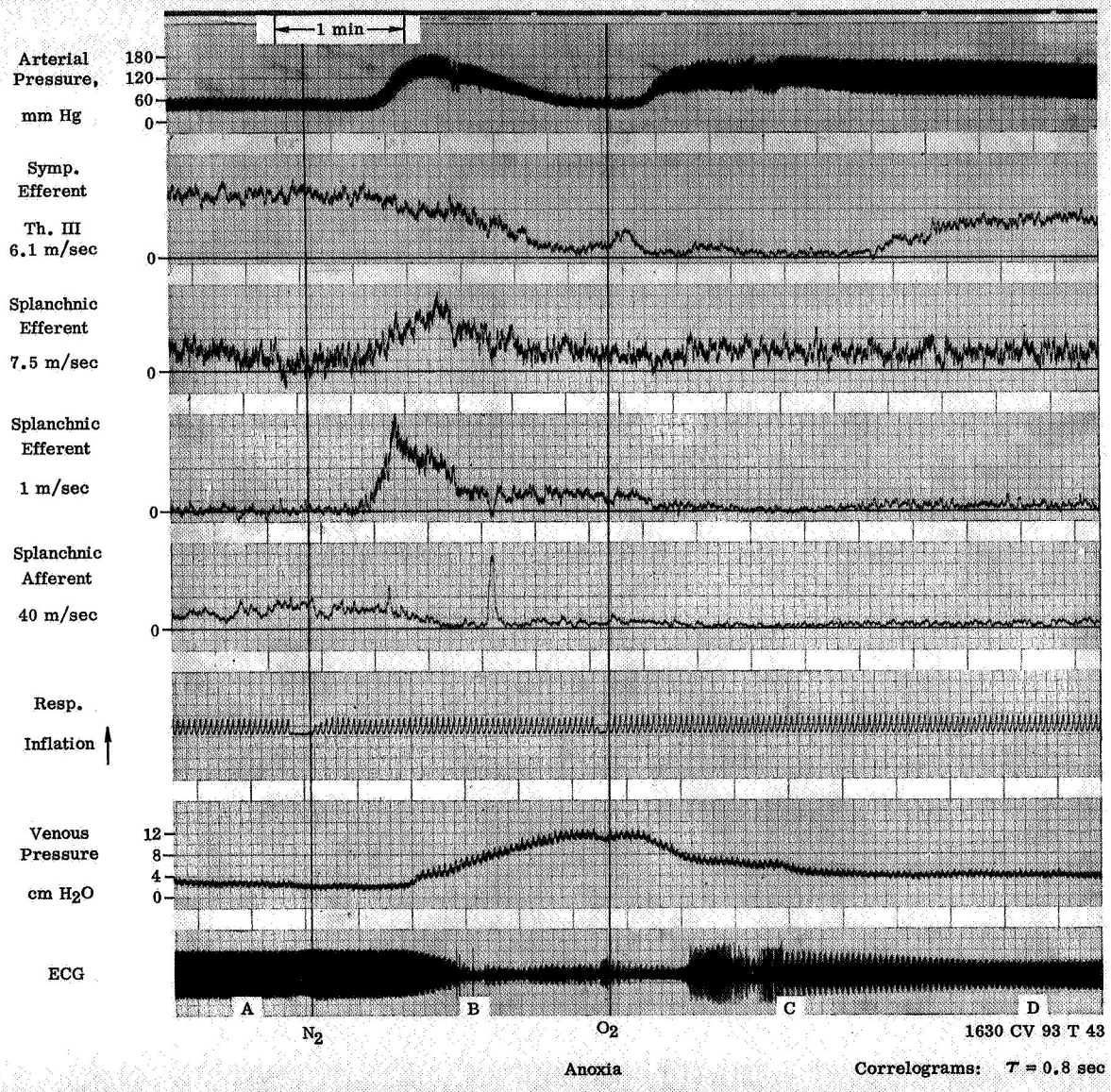


Figure 6. Response to a Period of Anoxia

At what level (or levels) in the nervous system hypoxia exerts its depressant effect cannot be deduced from the present experiments. The general rule is that the susceptibility to hypoxia within the central nervous system increases in the ascending direction. With respect to the circulatory centers, the evidence is that the direct effect tends to be one of depression, - which is, however, more or less masked by the stimulating action of signals arriving from the chemoreceptors. The relationship between degree of activity and hypoxic vulnerability as well as the apparent segmental differentiation, are phenomena which could be attributed to the functional organization of the medullary centers. It is, on the other hand, conceivable that sympathetic outflow may undergo drastic modification at the spinal cord segmental level - a possibility of considerable theoretical and practical interest.

The respiratory and/or cardiac cycle modulation of sympathetic outflow deserves comment. The tendency to oscillatory behavior is accentuated by the presence of CO_2, seemingly by increasing the neural output and by shortening the time-constant involved. The exact doubling of frequency (respiratory) seen during graded hypoxia in the presence of 7 1/2 percent CO_2, Figure 5, is relevant. (Respiratory rate was kept constant in the curarized animal.) The possible significance of modulated output patterns is at this point speculative - whether one relates it to the properties of the regulatory centers or to the effects on the peripheral target organs.

Conclusions. - In the acute experimental situation, sympathetic outflow in one thoracic segment may differ from that in another. During anoxia this differentiation may become accentuated; in an extreme, depression is seen in one segment while, at the same time, the outflow is increased several fold in another.

In the sympathetic response to anoxia, the pre-existing level of activity (segmentally) appears to be an important factor: if the level is high, e.g. as caused by hypoxia, anoxic depression is more likely to occur.

Increased tensions of CO_2 augments sympathetic outflow both in the normal and in the oxygen-deprived animal. Carbon dioxide exerts a profound and characteristic effect on the pattern of outflow - tending to accentuate its oscillatory behavior.

Adrenal Medullary Catecholamines and Circulatory Responses to Hypoxia

Originally, these experiments (total 24) were designed to explore the possibility of relating splanchnic nerve traffic to the output of catecholamines from the adrenal medulla. Although in some experiments the neural and the hormonal changes were quantitatively similar (as described in previous reports), in other experiments significant discrepancies were observed. Evidently at the present time our neuro-analytical methods are not selective and sensitive enough to pinpoint the fiber groups involved in the release of catecholamines.

The similarities between sympathetic ganglia and the adrenal medulla suggest that the dc potential over the gland might change as a consequence of activity, i.e., release of catecholamines. In the few measurements made, changes did occur (e.g., during anoxia or asphyxia), but in a rather unpredictable fashion. Data from one of the experiments are reproduced in Figure 7. In response to asphyxia (introduced by stopping the respirator used to ventilate the curarized animal), small shifts of dc potential were observed in addition to transient oscillations. The introduction of drugs (e.g., succinylcholine) would cause dc shifts, which however, were unpredictable as to direction and degree. Conceivably, improved methods might provide less ambiguous results.

Throughout the series of catecholamine experiments, graded hypoxia was frequently used as a challenge. The individual response to hypoxia was strikingly unpredictable - both with respect to catecholamine output and to circulatory behavior - and without any obvious connection between the two. Further analysis of the data, however, indicated a rather interesting trend.

Briefly summarized, in the data pooled from nine of the experiments, no clear correlations were found between mean arterial blood pressure, degree of hypoxia and total catecholamine output (Figure 8). However, when mean pressure was plotted against norepinephrine-epinephrine ratio (Figure 9), a trend became apparent: the higher the ratio, the higher the pressure. Possibly fortuitously, the ratio of unity coincides with a mean pressure of about 110 mm Hg.

If the just described trend should be substantiated in further experiments, it would raise the question of cause and effect: does the lowering of arterial pressure cause the relative increase in epinephrine or vice versa? Or, are they both functions of some other parameters?

Whatever the relationships are, it seems of great theoretical and practical interest to find out. The contrast between animals (cats) with low and high circulatory resistance to hypoxia is striking; similar individual variations may exist in man.

Monitoring of Baroreceptor Signals

Baroreceptor signals, originating in the aortic arch, are transmitted via the left depressor nerve to the brainstem of the central nervous system. In the neck (of cat and dog) this nerve usually is a part of the vagus nerve, and is thus contained within a common sheath (epineurium) together with several other nerve bundles. By cross-correlating the signals obtained from the vagus trunk, it is frequently possible to separate the traffic in the depressor fibers from that in other fiber groups.

Experimental Data. - Output from the aortic baroreceptors has been monitored in chronic and in acute experiments.

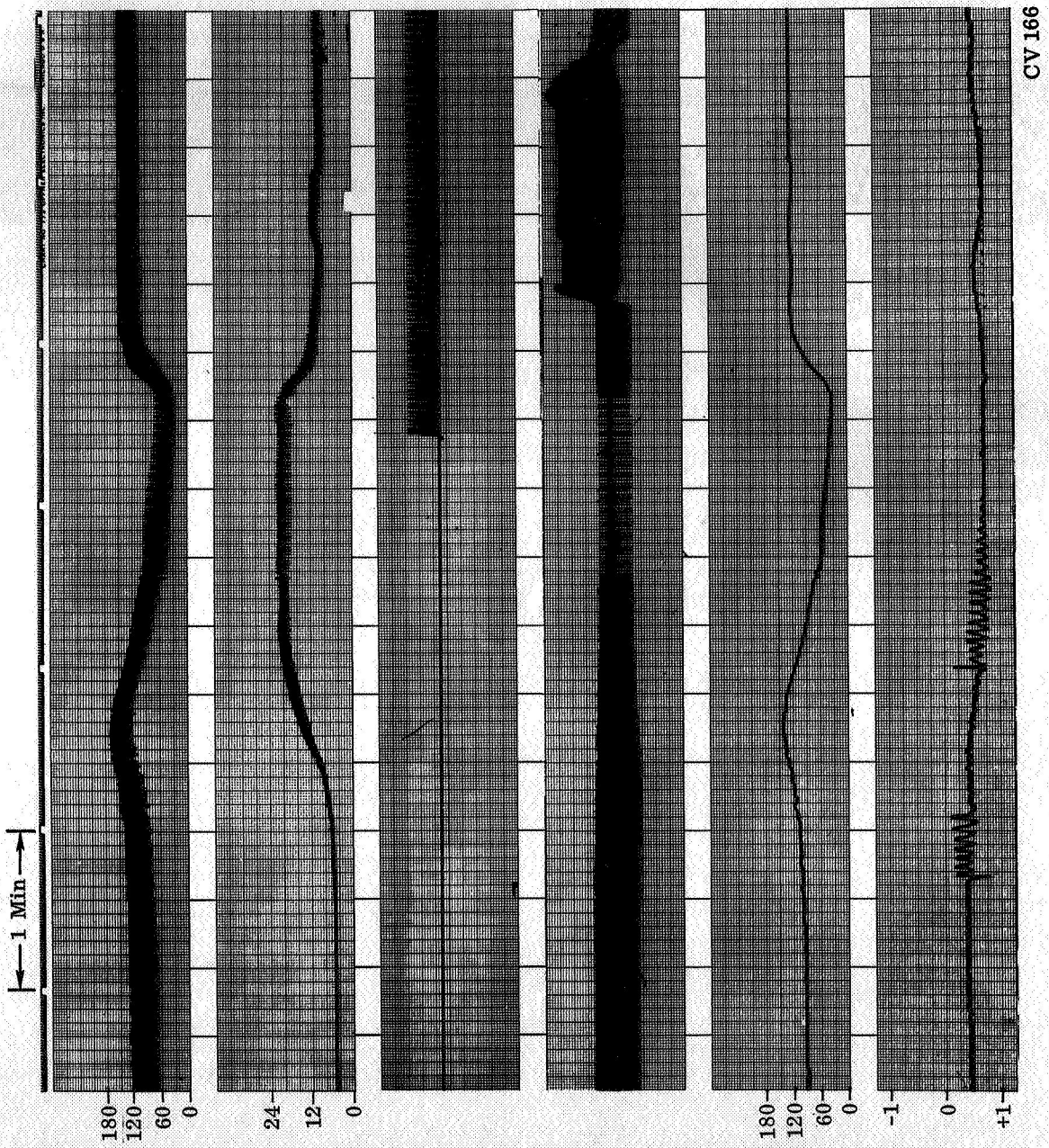


Figure 7. DC Potential Change as a Consequence of Release of Catecholamines

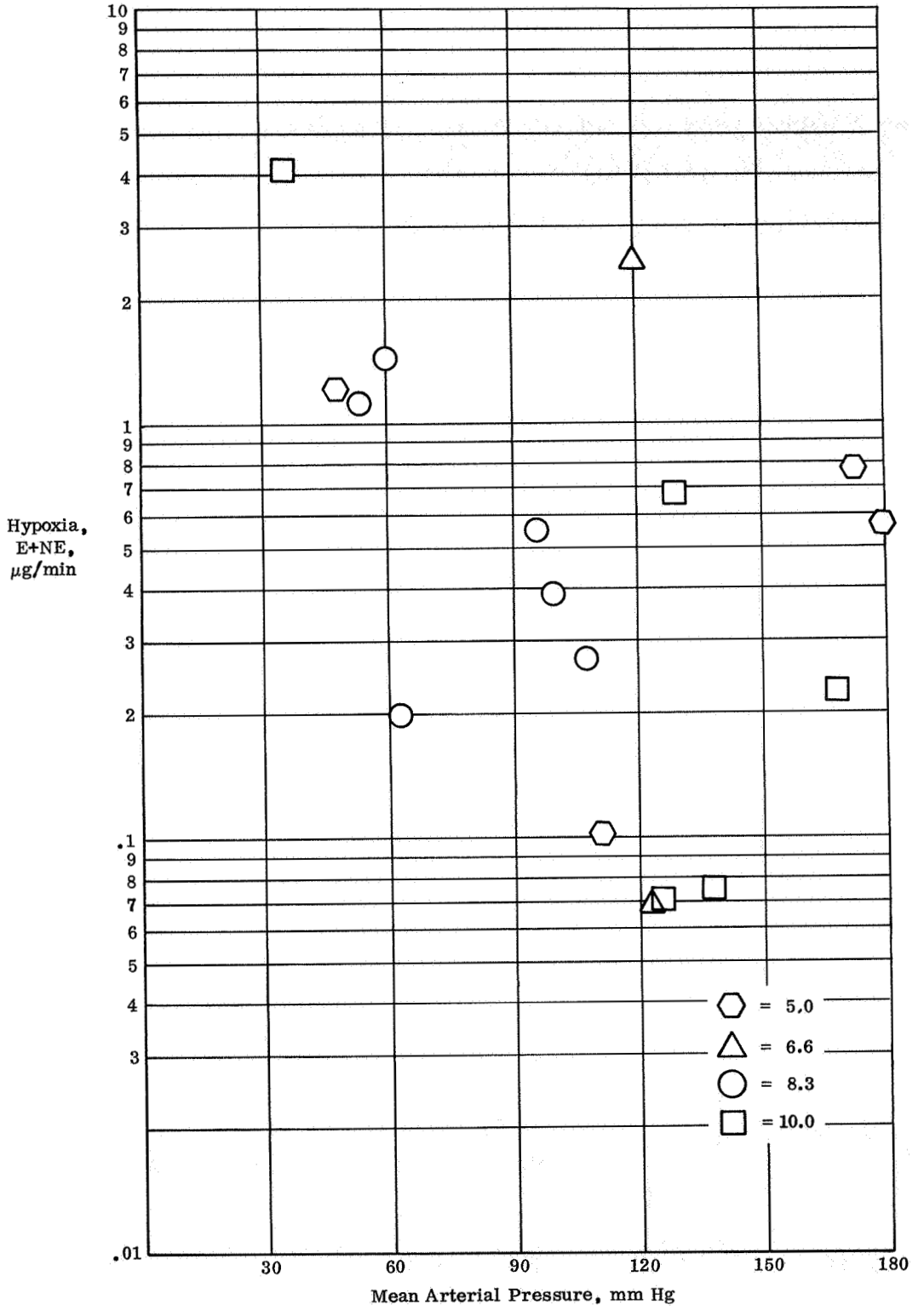


Figure 8. Data Pooled From Nine Experiments

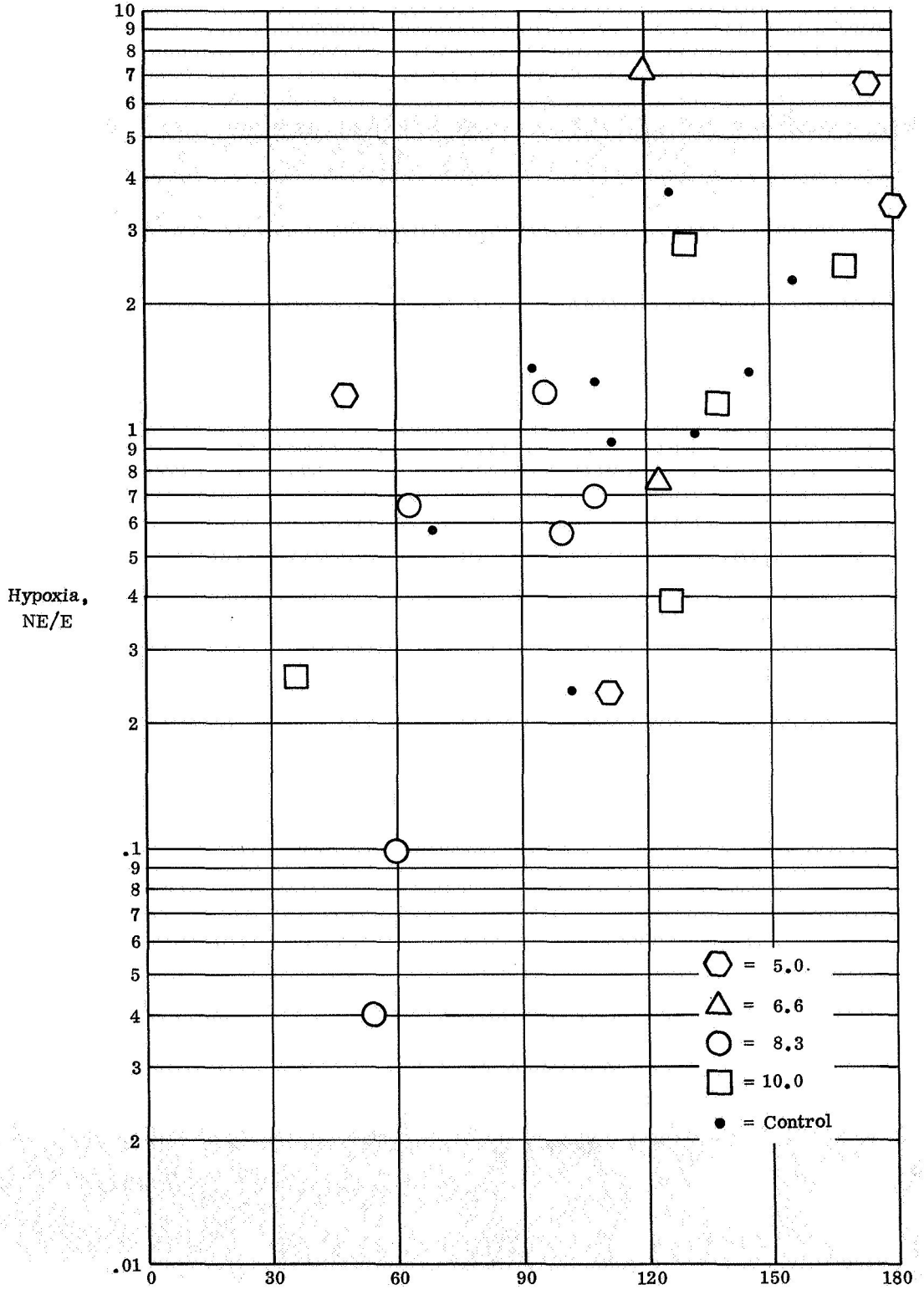


Figure 9. Mean Pressure Versus Norepinephrine-epinephrine Ratio

Chronic experiments: The vagus nerve in the neck (particularly in the cat) lends itself to chronic, i.e., electrode implantation experiments. The nerve is easily accessible, and the pulsatile output pattern facilitates testing.

In Figures 10 and 11 are reproduced data from a representative experiment. The data in Figure 10 were obtained shortly after implantation of electrodes, the animal still being under the influence of anesthesia and surgery. Cross-correlated data,seen in the left panel, was fed into the CAT - and the result of averaging is displayed in the right panel. On the fifth day after surgery, utilizing identical data processing, the records reproduced in Figure 11 were obtained. By gross inspection it is evident that the changes in the central arterial pressure are in a sensitive manner reflected in baroreceptor output patterns.

Acute experiments: The purpose of the experiment depicted in Figures 12 and 13 was to examine the question whether or not the transfer-function of the aortic baroreceptors is constant. The reasoning behind this goes as follows: baroreceptors are basically sensing stretch (distortion) of the vessel wall. The wall of the aorta contains a certain amount of smooth muscle, innervated by sympathetic fibers. Consequently, the possibility exists that sympathetic outflow, by changing the wall characteristics, may alter baroreceptor transfer-function. In Figure 13 is seen the result of ganglionic block induced with Arfonad. The purpose of Arfonad administration was to reduce sympathetic "tone" by ganglionic block. The ensuing hypotension is seen in the right panel of Figure 13. In order to create conditions (in the aorta) comparable to those existing during the control period, a snare was placed around the aorta just below the diaphragm. On tightening the snare, the pressure in the aorta rose to levels comparable to the control values. From the data obtained during aortic clamping, a section was selected in which baroreceptor output most closely matched that seen during the control period - as seen in Figure 12, right and left panel respectively. It appears that under conditions of ganglionic block and a clamped abdominal aorta, less intra-aortic pressure is needed for the same baroreceptor output. (Considering the rate sensitivity of the receptors, it should be noted that the derivative of the pressure was similar under the two conditions.)

Information concerning arterial pressures reaches the brain from receptors located in the aortic arch, right subclavian and right common carotid arteries, the carotid sinuses, and the peripheral arterial tree. The sinoaortic are assumed to be the major contributors under most circumstances.

Since the arterial pressure wave travels at a speed lower than that of neural conduction, it is likely that the centers in the brain will "see" information arriving from the aortic and sinus receptors at somewhat different times. In the experiment depicted in Figure 14, nerve traffic was recorded simultaneously from the left depressor and the left carotid sinus nerves, and displayed on an oscilloscope screen, the sweep being triggered by the R wave in the ECG. Picture "A" shows the bursts of nerve impulses as they were recorded, and picture "B" shows the appearance after cross-correlation (vagus) and integration (1 msec time-constant). It will be seen that the delay between

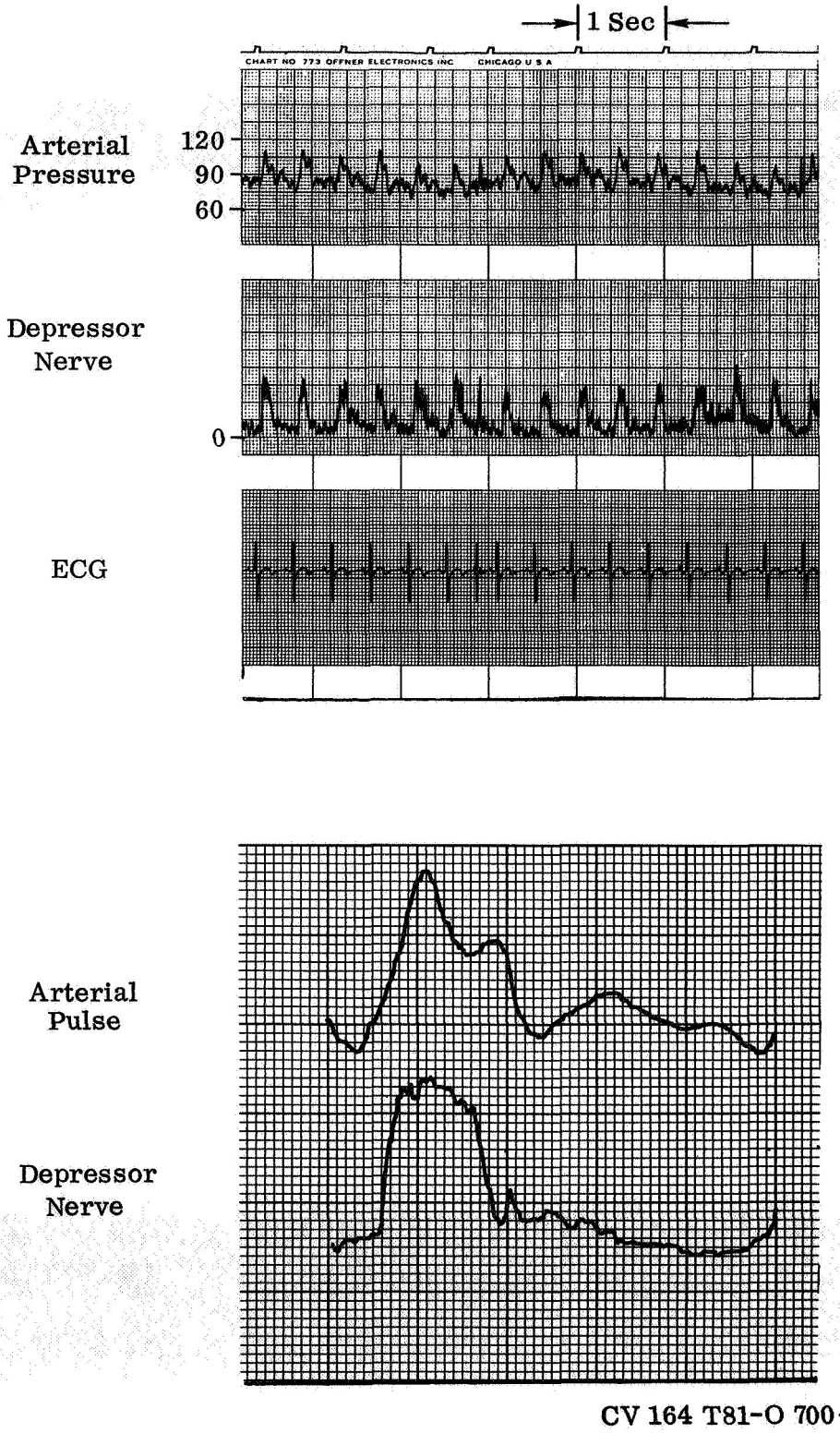


Figure 10. Output From Aortic Baroreceptors Shortly After Implantation of Electrodes

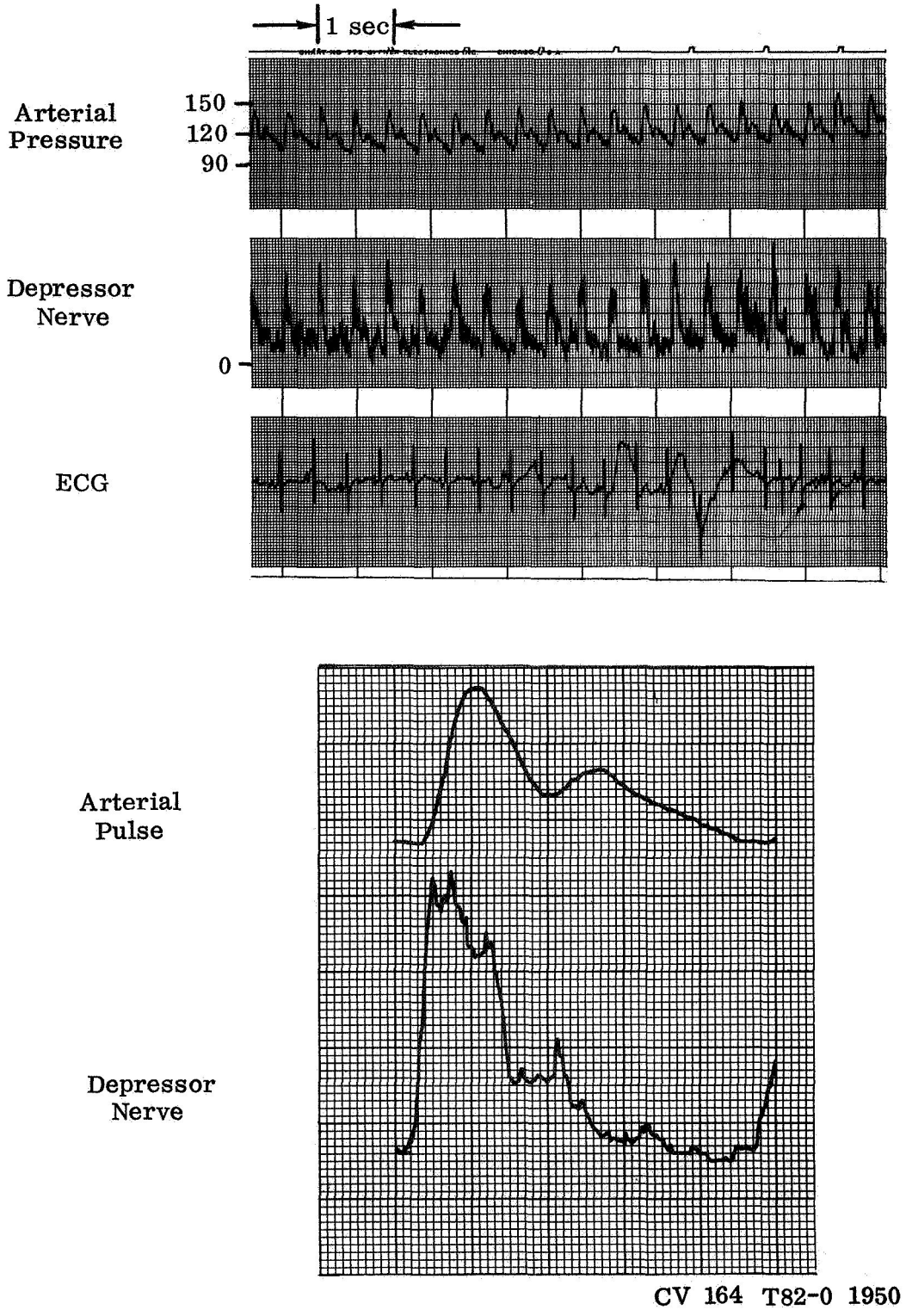


Figure 11. Output From Aortic Baroreceptors Five Days After Surgery

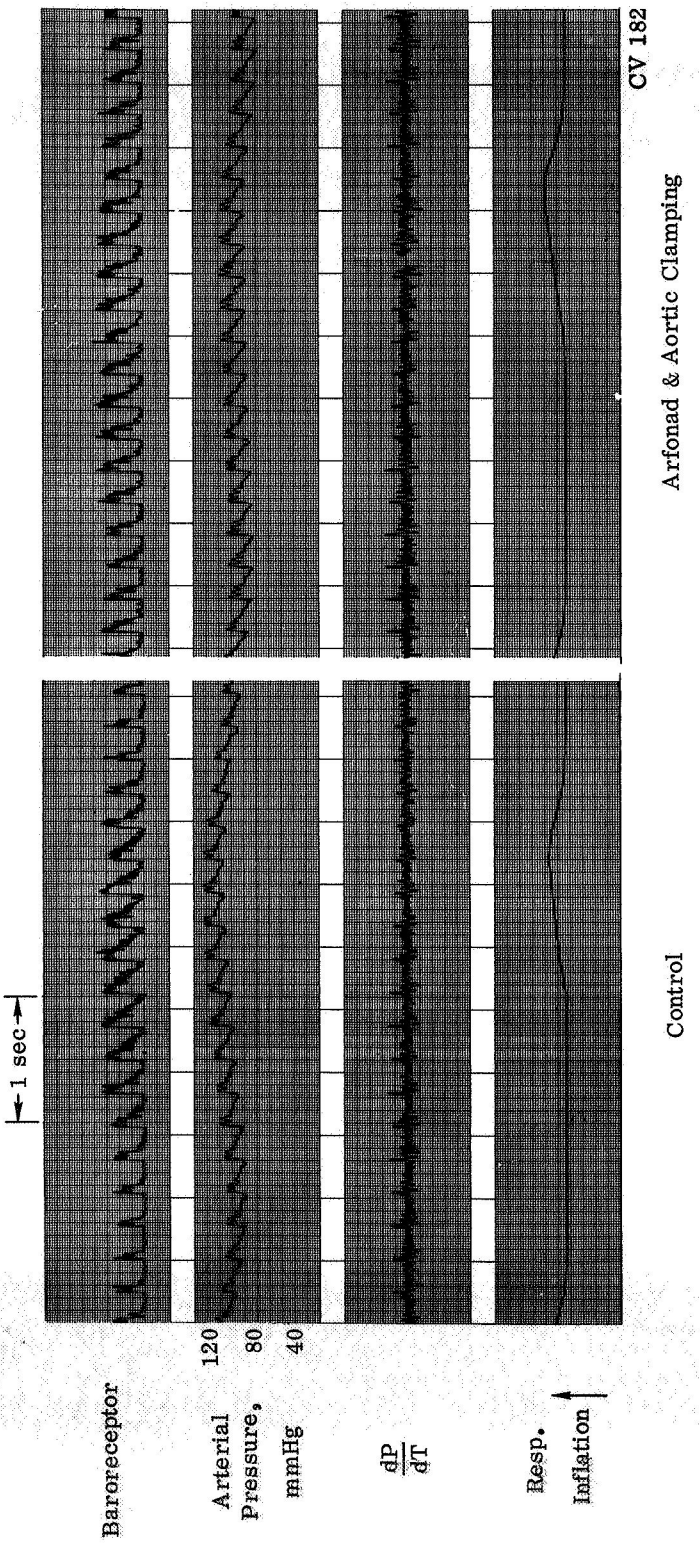


Figure 12. Result of Ganglionic Block Induced With Arfonad and Clamped Abdominal Aorta

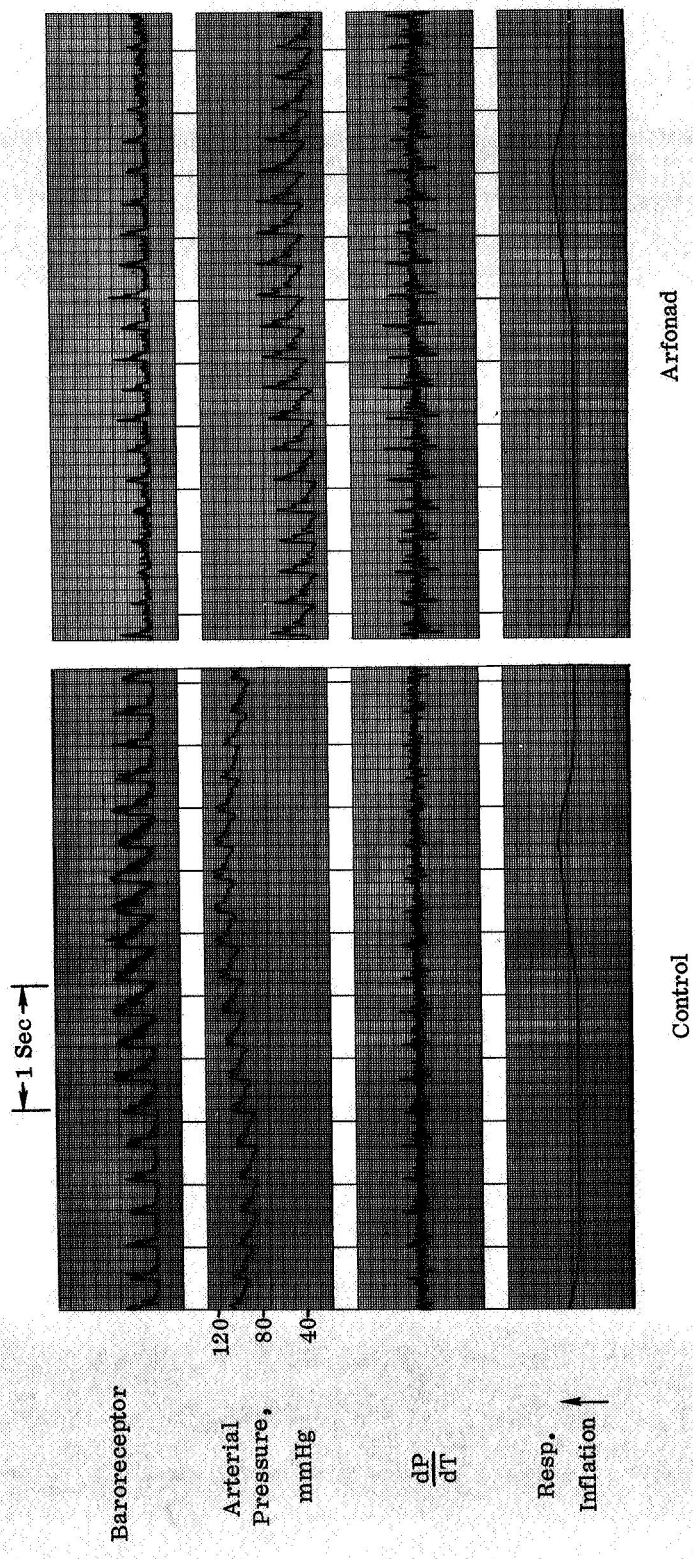


Figure 13. Result of Ganglionic Block Induced With Arfonad

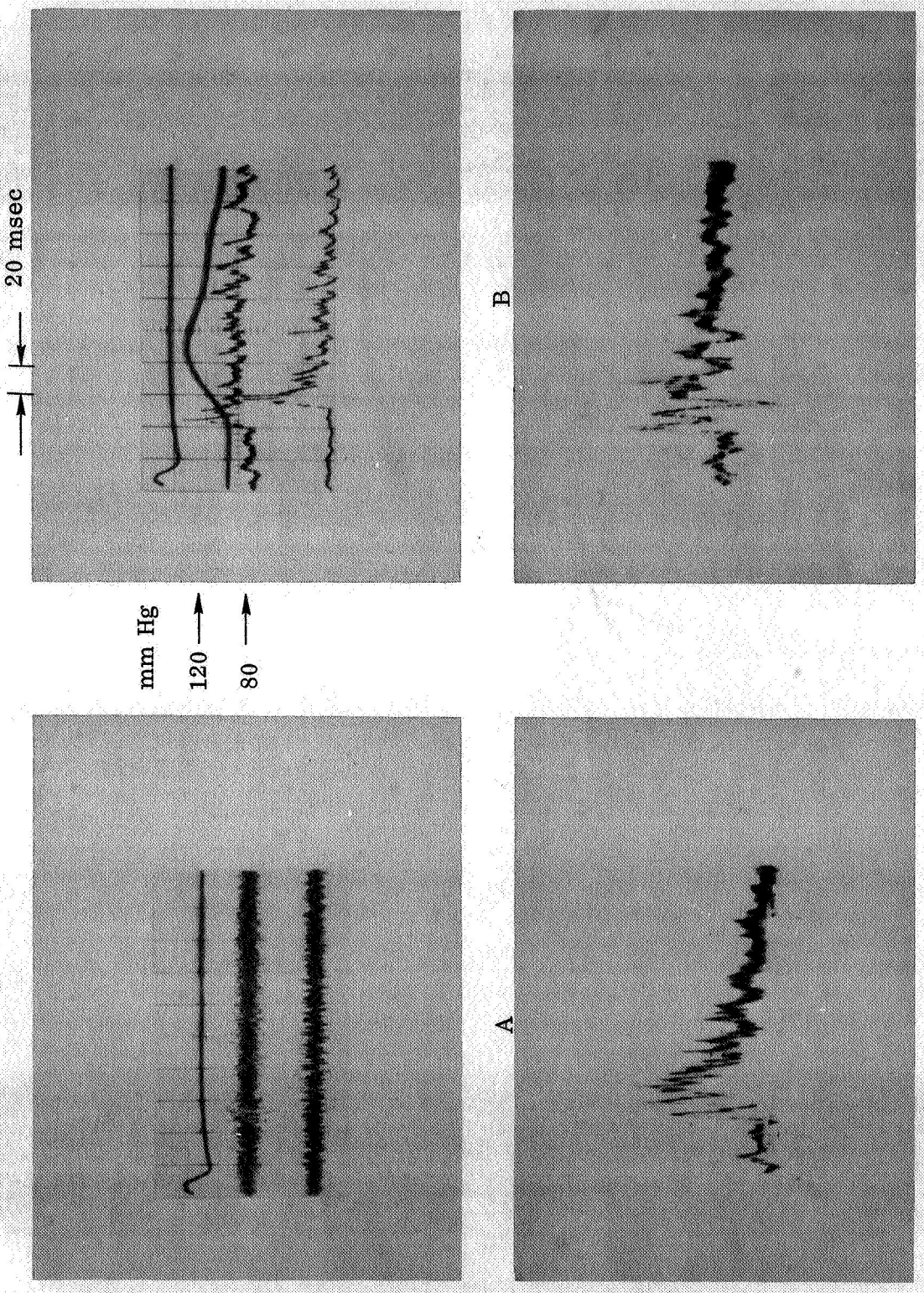


Figure 14. Left Depressor and Carotid Sinus Nerve Activity

aortic (upper nerve trace) and sinus signals was about 12 msec at the "foot" and 15 msec at the peak. Adding the two signals algebraically resulted in the waveform displayed in picture "C". Picture "D" shows the result of adding the sinus signal at reversed polarity to the aortic signal.

Discussion and Conclusions. - The main value of the chronic experiments has been the experience gained in electrode implantation methodology. A base has been lain for future studies when necessary hardware can be gotten hold of (such an implantable devices for a blood pressure and flow measurements). The important role played by the baroreceptors in cardiovascular control needs further elucidation.

The results of acute experiments support the thesis that the relationship between intra-aortic pressure variations and baroreceptor "output" is not a constant one. Although obtained by indirect means, the evidence is that sympathetic "tone" does influence the "transfer function" - probably by its effect on anortic smooth muscle, as suggested by Palmer and by Peterson.

The simultaneous recording of baroreceptor impulses from the aorta and from the carotid sinus provided an accurate measure of pulse velocity along the cat carotid artery, namely 16.7 meters per second. It may be worth noting that if a summing of the two takes place in the brain centers, then both amplitude and temporal summation will contribute to the waveform seen by these centers.

Monitoring of Signals from Pulmonary Stretch Receptors

On the basis of what had been learned during the program, in technology and in physiology, it was proposed to the National Aeronautics and Space Administration that the properties of the baroreceptors and their functional significance be made the subject for continued study; these "feedback" signals are intimately involved in the performance of the cardiovascular system. Late in the program it became evident that the means to pursue the proposed course of action would not be available. Consequently, attention was directed toward a related system, namely the pulmonary. With some improvisations, this could be explored with the means at hand.

The lungs and their ventilation are intimately related to cardiovascular function - not only with respect to gaseous exchange, but also by direct and reflex involvement in circulatory dynamics. The close neural connections between the two systems may be exemplified by the respiratory modulation of sympathetic outflow described earlier in this report.

The vagus nerve, in the neck, contains fibers whose signals originate in pulmonary stretch receptors. Functionally, the impulses concerned exert a profound effect on respiratory amplitude and rate (the "Hering-Breuer Reflex"). In suitable preparations (cat and dog) this afferent nerve traffic may be extracted from the vagus nerve.

Experimental Data. - A paper entitled "Neural Monitoring of Pulmonary Ventilation" was accepted for presentation at the 7th International Conference on Medical and Biological Engineering in Stockholm, August 14 - 19, 1967. A copy of the paper, as it appears in the Transactions from the meeting, is included as Appendix A.

Because of space limitations in the Transactions, the experimental data reproduced in Figure 15 were not included. The tracings were obtained by operating a sinusoidal respiratory pump (curarized cat) at varying frequencies. In each section the upper tracing represents neural output and the lower trace represents radial expansion of the chest. It will be seen that within the frequency range of 5.5 to 60 per minute the nerve pattern paralleled the variations in chest diameter, with little change in amplitude and without phase-shift.

Discussion of Results. - As with baroreceptor impulses, the signals from the pulmonary stretch receptors may be analyzed from two points of view: origin and destination. With respect to their orgin, one wants to know what the generated information represents; with respect to their destination (the central nervous system), one wants to know what happens to the information, i.e., its functional significance. As of this writing, we have had the opportunity to examine systematically only the first mentioned aspect.

Although it is well known that the sensors in question are of the slowly adapting type, it is rather remarkable the fidelity with which the nerve pattern mirrors the radial expansion of the chest. When compared to the results obtained by other investigators, the main experimental difference is that in the present experiments the recorded activity is derived from a population of fibers and not only a few. Thus, contributing to the overall envelope,will be both the number of fibers activated and the frequency of impulses in each fiber.

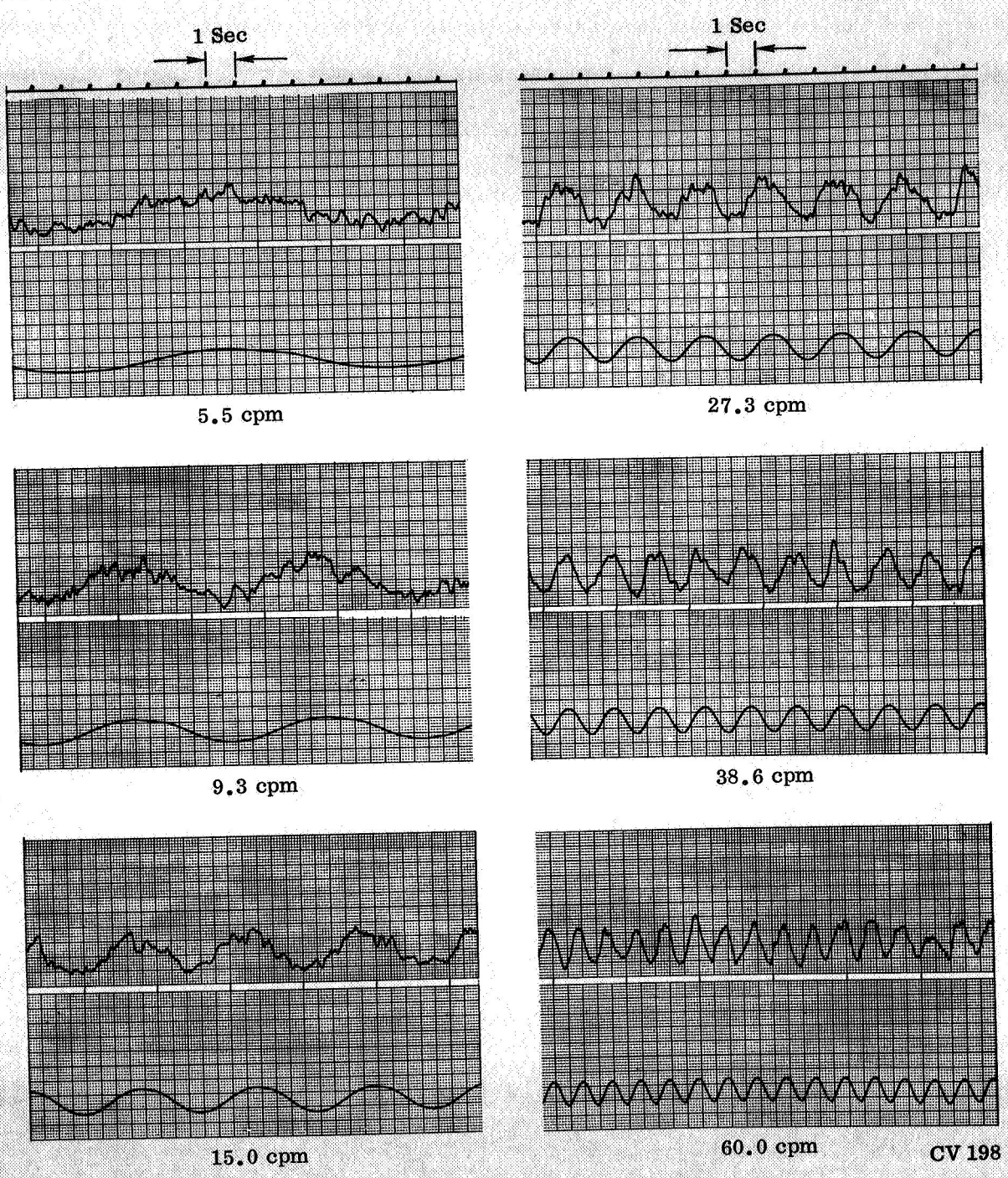


Figure 15. Frequency Response of Pulmonary Stretch Receptors

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HSER 5004
National Aeronautics and Space Administration
EXPERIMENTAL AND ANALYTICAL STUDY OF CARDIOVASCULAR CONTROL LOOPS
N. A. Normann, M. D.

Neuro-humoral factors are intimately involved in control of the circulation of blood. The ability of the cardiovascular system to respond appropriately to sudden changes and to increased demands is to a great extent due to the existence of neural control loops - providing system adaptation with suitable speed (time-constants) and stability.

The neural, autonomic* pathways involved and their functional significance are well known in a general, qualitative way. In contrast, knowledge of the quantitative, operational characteristics of the autonomics in the intact organism is rather scant; conclusions in this regard are reached mainly by interpretation of "symptoms", i.e., by inference. This lack of direct, pertinent information pertains both to "normal" and "abnormal" states; knowledge of the exact mechanisms involved in circulatory adaptive processes is fragmentary - whether the adaptation is in the response to a coronary occlusion or to a change in gravitational load. Observations of hemodynamic quantities, such as pressures and flows are by themselves apt to be of limited value, inasmuch as they represent parameters which the system tends to control. Direct access to information flowing in relevant control loops would be expected to produce further insight into system behavior and might also provide a sensitive, quantitative indicator of certain adaptive processes.

I. N. A. Normann, M. D.
II. HSER 5004

NASA

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APPENDIX A
NEURAL MONITORING OF PULMONARY VENTILATION
(PAPER PRESENTED AT STOCKHOLM CONFERENCE)

TUESDAY, AUGUST 15, AFTERNOON HALL E
SESSION 12 NEUROPHYSIOLOGY

12–6 Neural Monitoring of Pulmonary Ventilation

N. A. Normann
Bio-Sciences and Technology Department
Hamilton Standard Division
United Aircraft Corporation
Windsor Locks, Connecticut, U.S.A.

M. A. Bianchi
Bio-Sciences and Technology Department
Hamilton Standard Division
United Aircraft Corporation
Windsor Locks, Connecticut, U.S.A.

A. T. Gelman
Bio-Sciences and Technology Department
Hamilton Standard Division
United Aircraft Corporation
Windsor Locks, Connecticut, U.S.A.

The vagus nerve contains fibers which carry signals originating in pulmonary stretch receptors. It has been shown that the impulse frequency in the individual fibers increases with expansion of the lungs and the trachio-bronchial tree.[1,2] The purpose of the present study is to examine the feasibility of extracting this sensory information from the intact vagus nerve and to establish the relationship between the impulse patterns and pulmonary ventilation.

Methods. In anesthetized cats, the right vagus was exposed in the neck. Two pair of electrodes were placed on it. After amplification, the two channels of nerve signals were recorded on tape, together with the data from the following measurements: Pulmonary expansion was measured by utilizing a spirometer and by transducers sensing the movements of chest and abdominal walls. Multiplied by suitable coefficients, the latter quantities were electronically added to give the sum of wall movements. Air flow was measured with a hot-wire anemometer. Played back from the tape recorder, the nerve signals were analyzed by cross-correlation, and the resulting impulse pattern was displayed on a real time basis together with the transducer data.

Results. In most cats weighing less than 2½ kg, nerve traffic from pulmonary stretch receptors can be obtained from the intact vagus without splitting the epineurium. Results of cross-correlation analysis indicated maximum activity in fibers conducting impulses at speeds of 35 to 45 meters per second. A near linear relationship was found between the amplitudes of summed wall movements and of neural activity (Fig. 1). In the curarized animal, the linear relationship appeared to hold over a wide range of controlled amplitudes (Fig. 2).

Discussion and Conclusions. The conduction velocities of the fibers monitored belong to a group which is dominated by slowly adapting fibers.[3,4] In response to a sinusoidal inflation (curarized animal), 5 to 60 cycles per min., the waveform of the nerve traffic parallels that of the chest-abdominal walls, without phase shift and with little change in amplitude. The linear relationship between the combined axes ("radii") of the chest and the power density of the nerve impulses disagrees with Adrian's results, but is consistent with those of Kiddicombe (an approximately linear proportionality between cube root of his volume values and impulse frequency). As an extension of this approach, electrodes have been implanted on the vagus, allowing monitoring in the intact, unanesthetized animal. In conclusion, it appears feasible to extract from the intact vagus information concerning pulmonary ventilation.

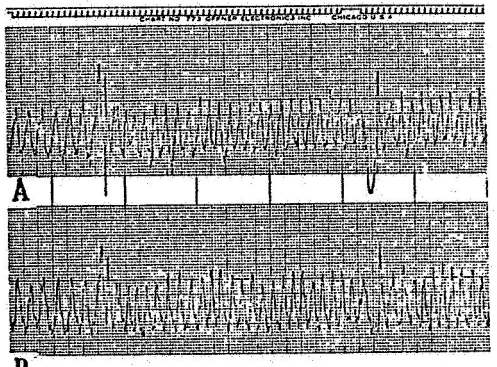

Fig. 1. Spontaneous breathing. A. Sum of two chest radii. B. Pattern in sensory nerve fibers. Time marker: min. and sec.

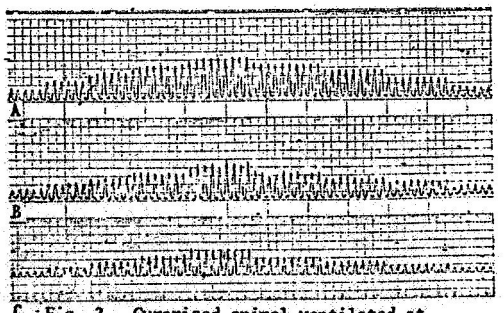

Fig. 2. Curarized animal ventilated at varying amplitudes of inflation. A. Sum of two chest radii. B. Pattern in sensory nerve fibers. C. Inflation pressure.

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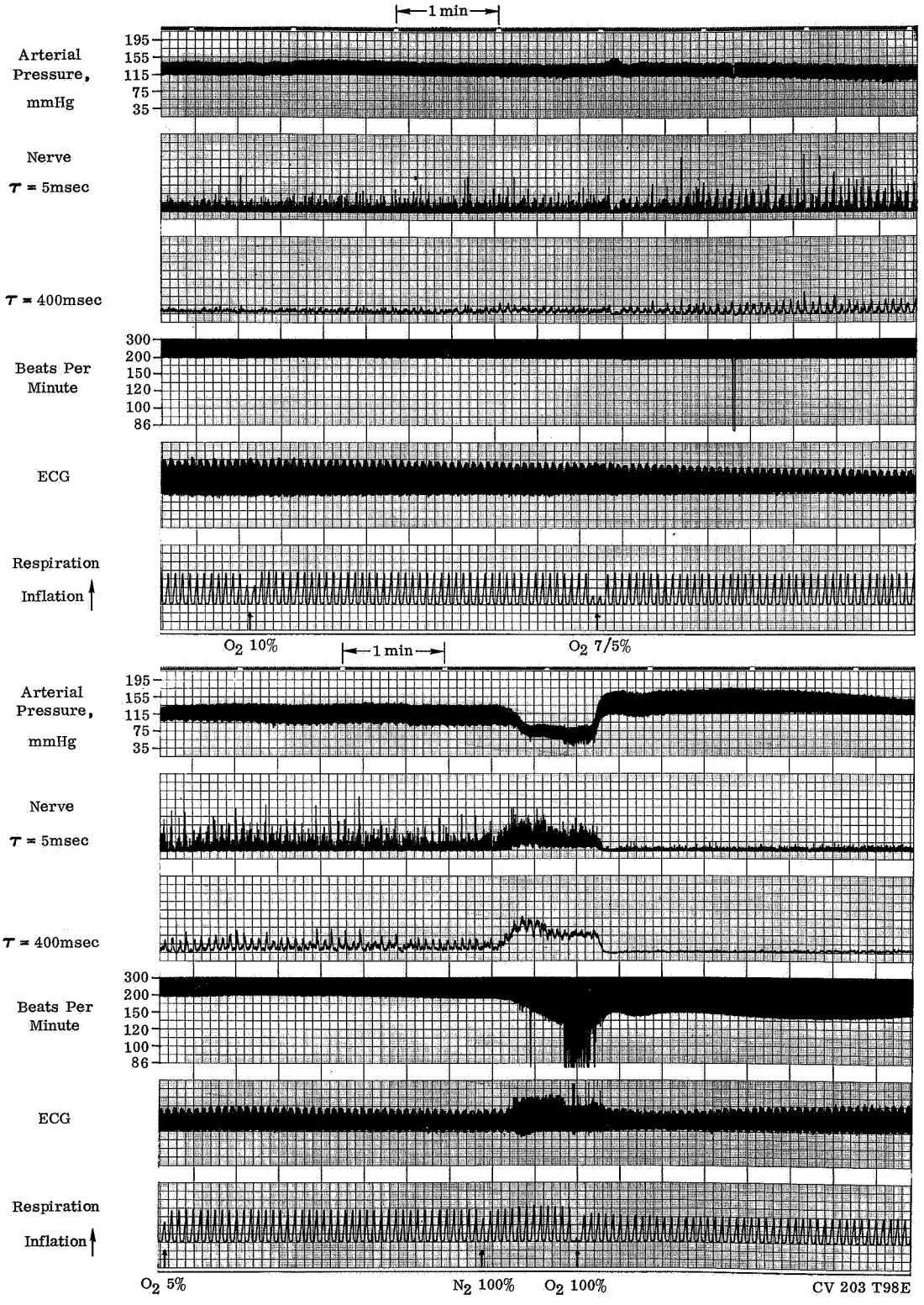
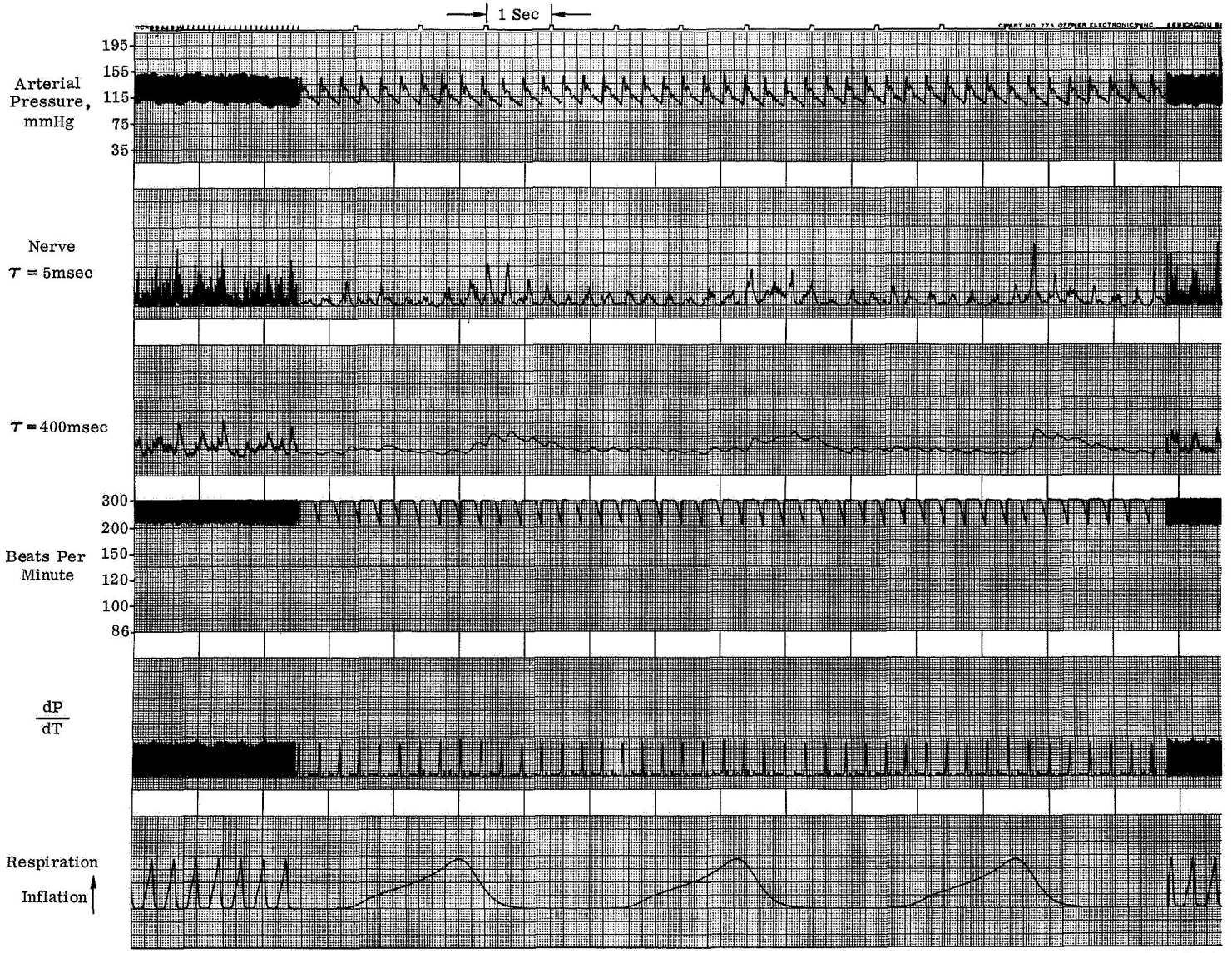
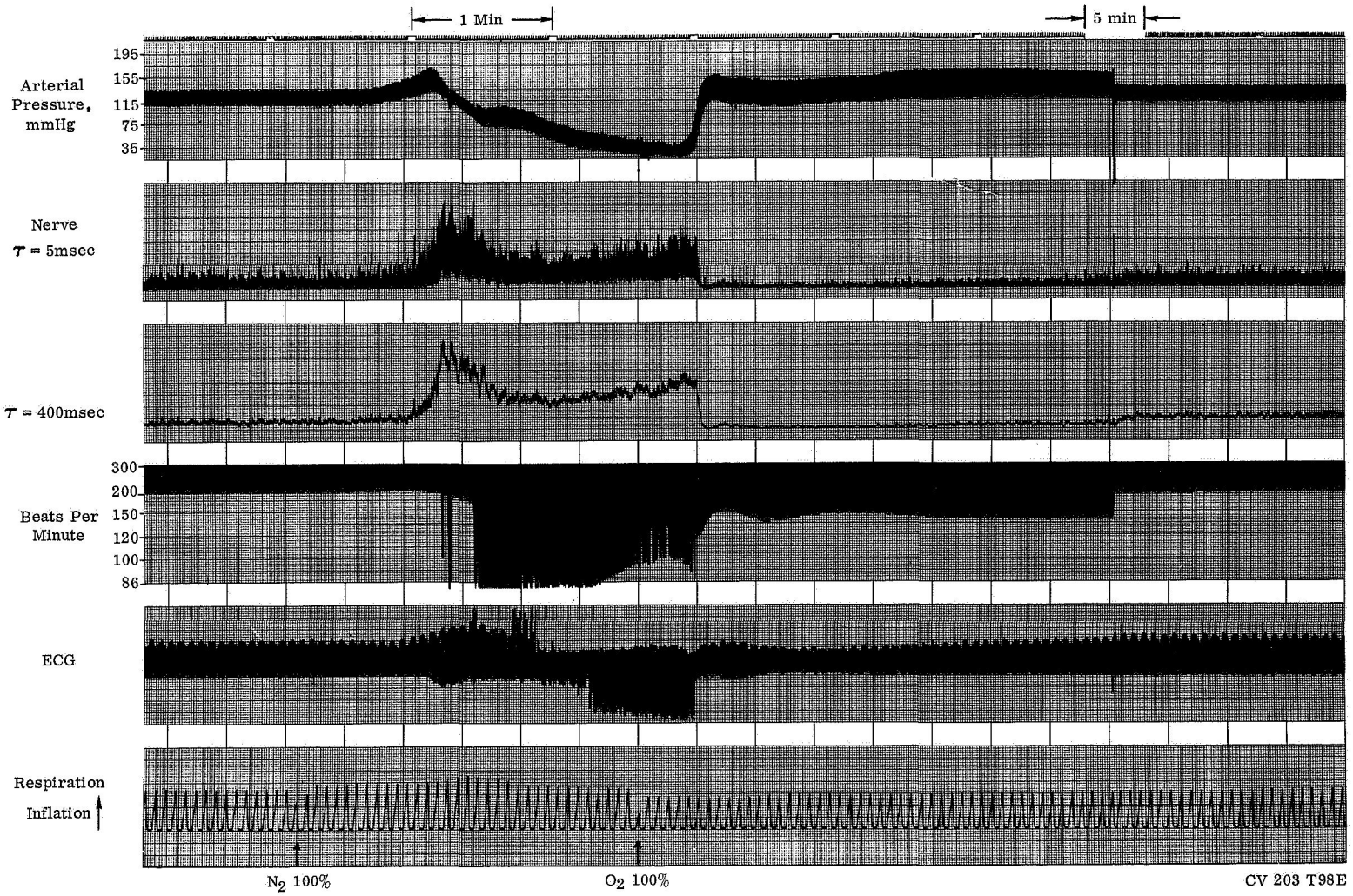
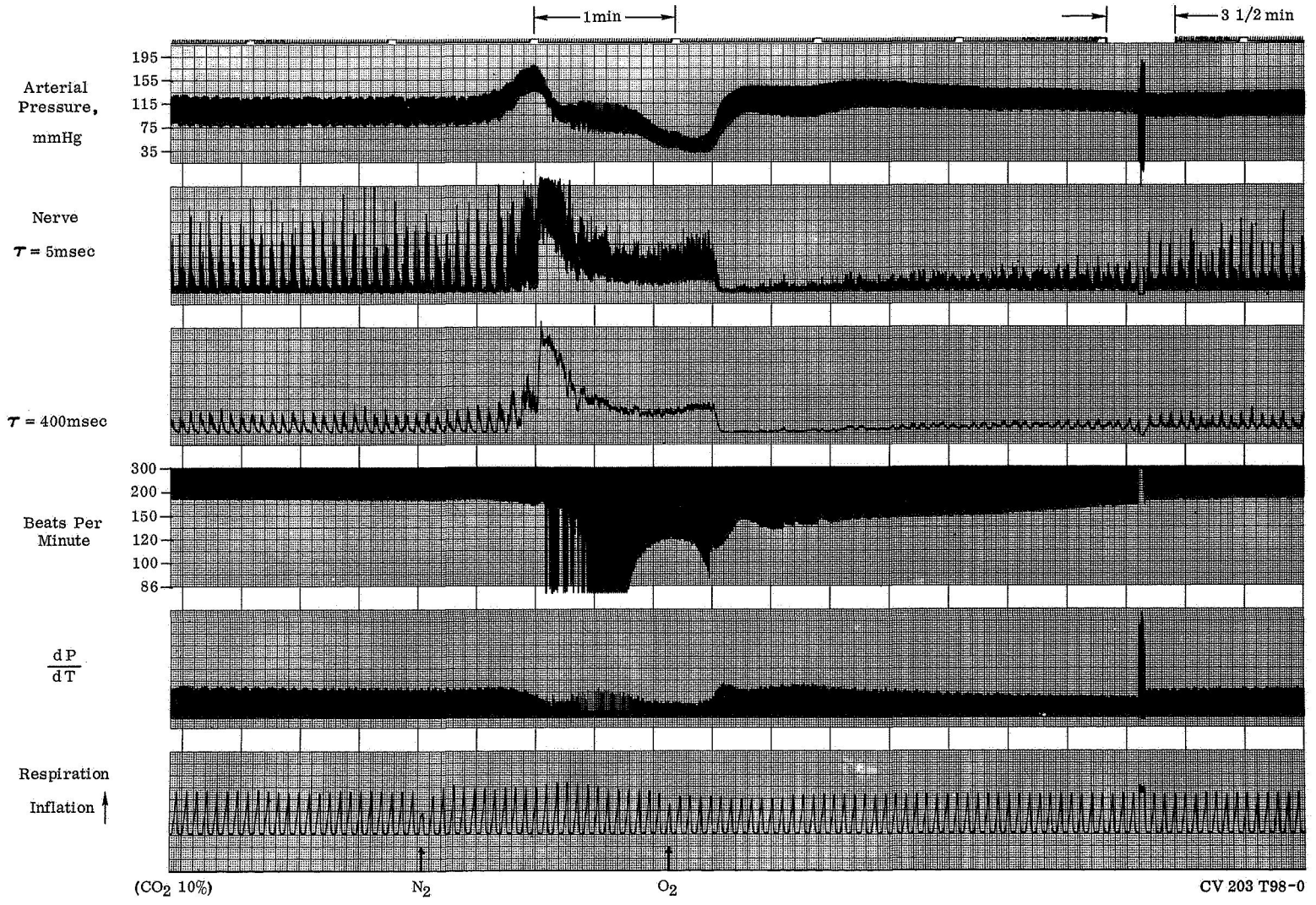
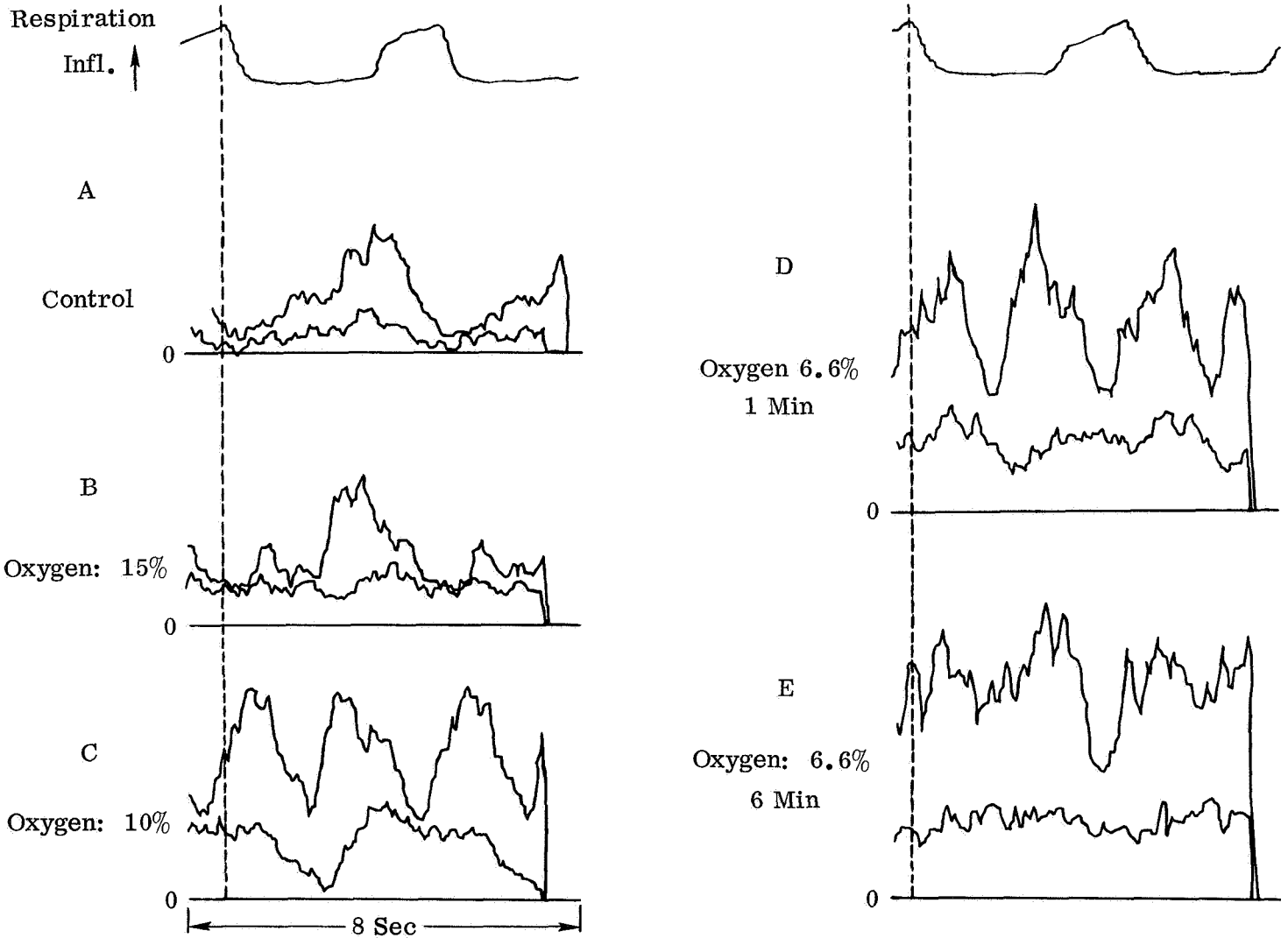

1 min
Arterial Pressure, mmHg
195
155
115
75
35
Nerve
τ = 5msec
τ = 400msec
300
200
150
120
100
86
Beats Per Minute
ECG
Respiration
Inflation
O2 10%
1 min
O2 7/5%
Arterial Pressure, mmHg
195
155
115
75
35
Nerve
τ = 5msec
τ = 400msec
300
200
150
120
100
86
Beats Per Minute
ECG
Respiration
Inflation
O2 5%
N2 100%
O2 100%
CV 203 T98E


1 Sec
Arterial Pressure, mmHg
195
155
115
75
35
Nerve
τ = 5msec
τ = 400msec
300
200
150
120
100
86
Beats Per Minute
dP/dT
Respiration
Inflation

CV203 T98E

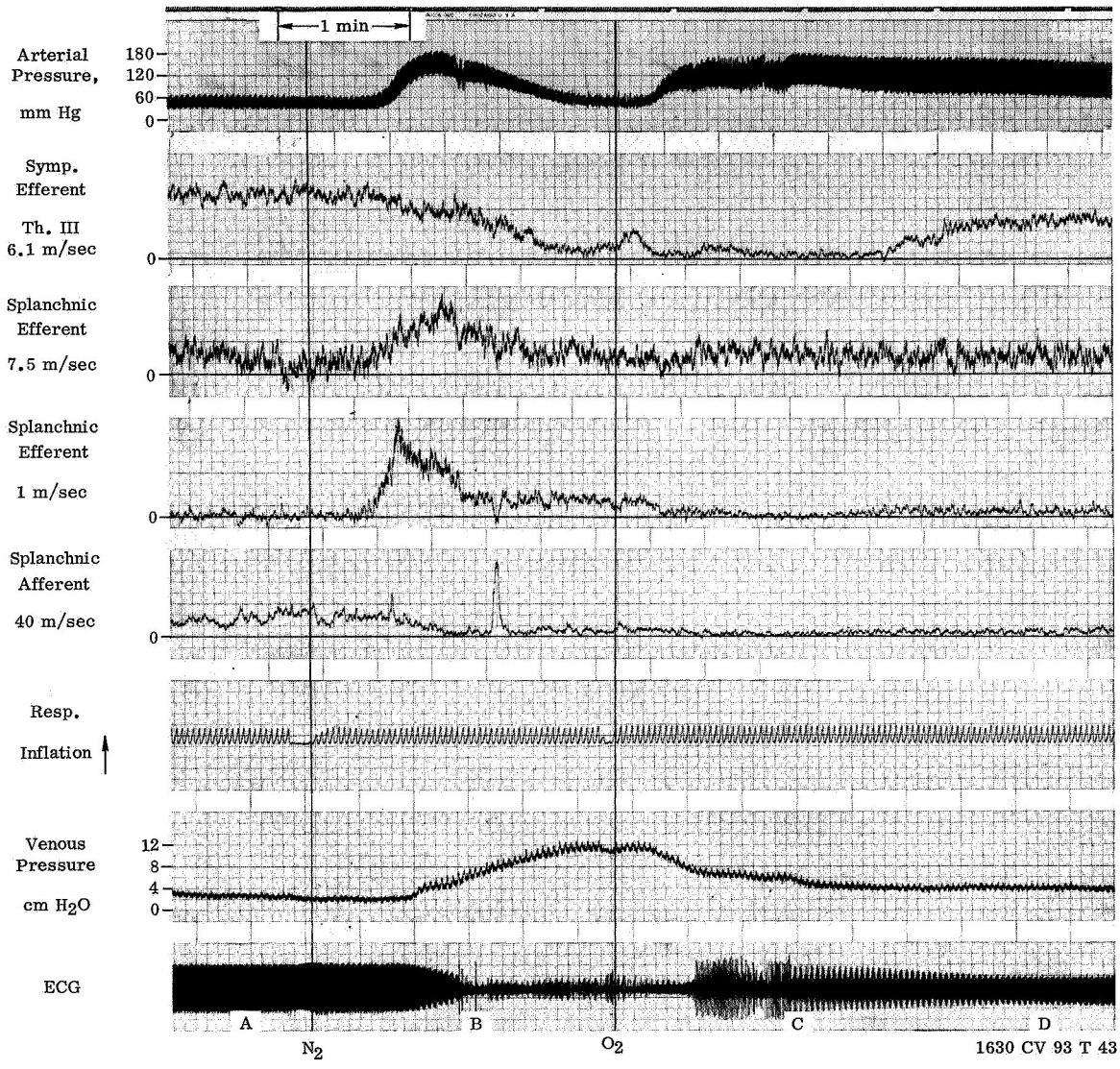

1 Min
5 min
Arterial Pressure, mmHg
195
155
115
75
35
Nerve
τ = 5msec
τ = 400msec
300
200
150
120
100
86
Beats Per Minute
ECG
Respiration
Inflation
N2 100%
O2 100%
CV 203 T98E


1min
3 1/2 min
Arterial Pressure, mmHg
195
155
115
75
35
Nerve
τ = 5msec
τ = 400msec
300
200
150
120
100
86
Beats Per Minute
dP/dT
Respiration
Inflation
(CO2 10%)
N2
O2
CV 203 T98-0



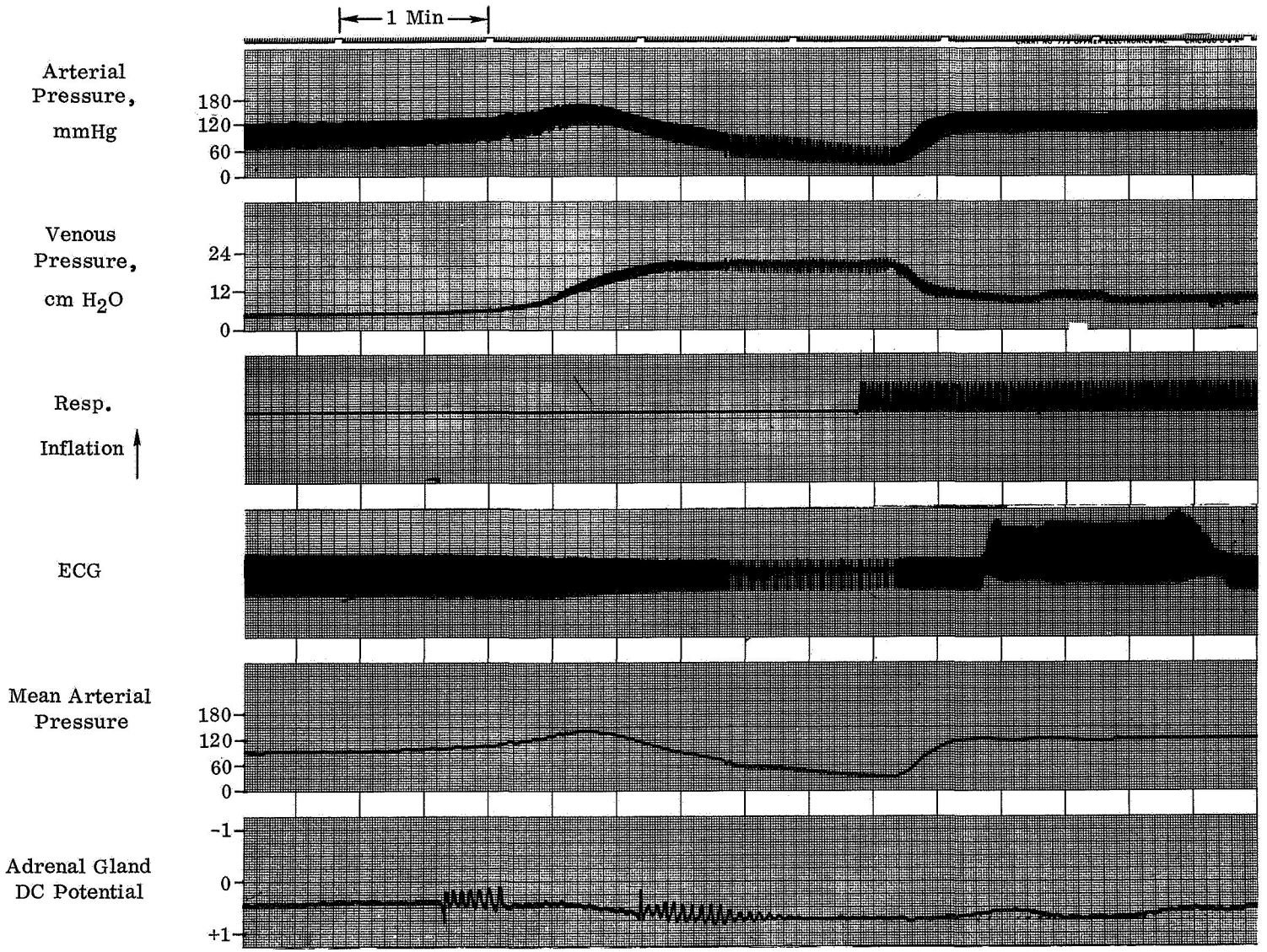
Sympathetic Outflow In Ninth Thoracic Ramus
During Hypoxia With CO_2 1% & 7.5%

CV 115 T64A-B

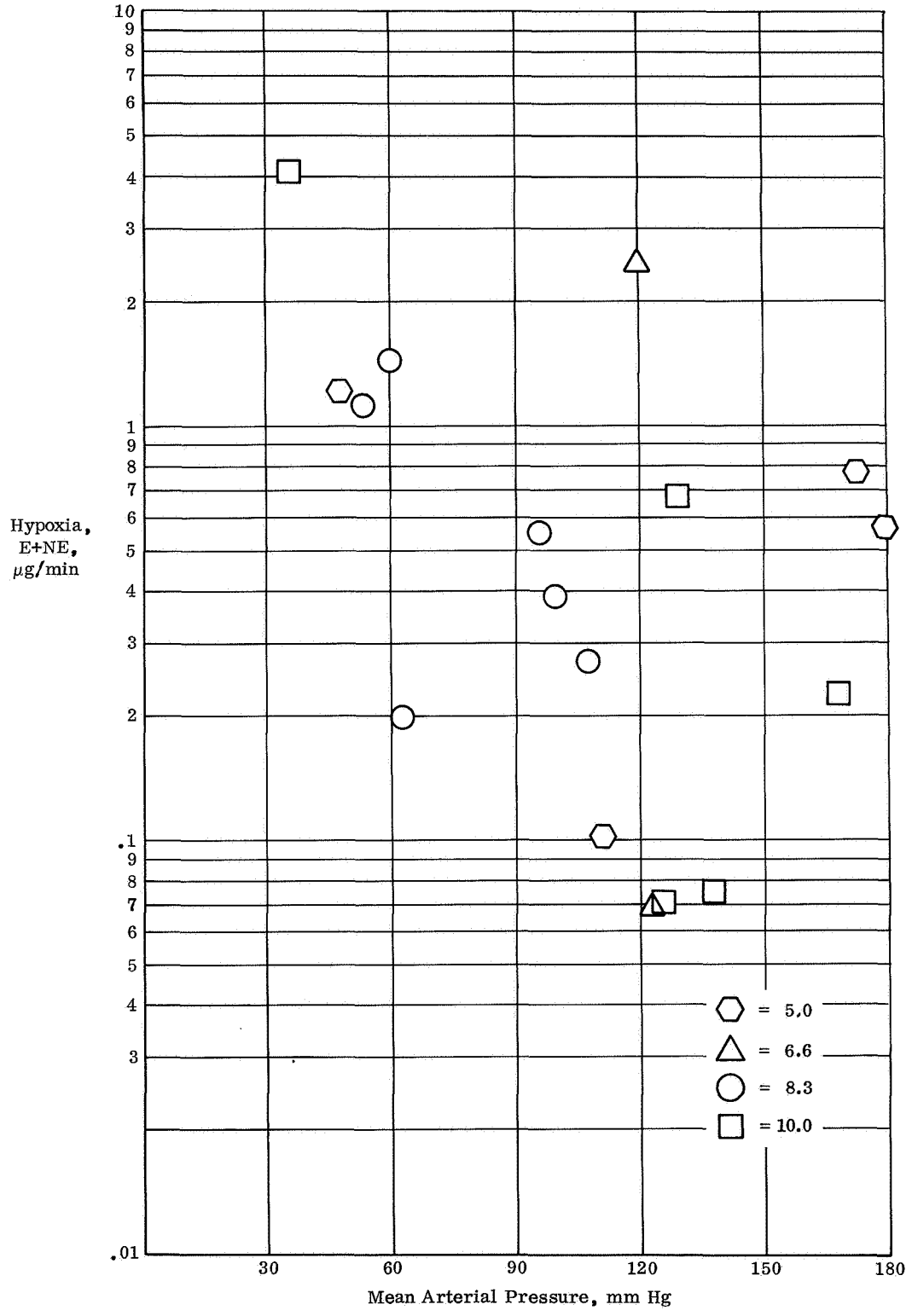


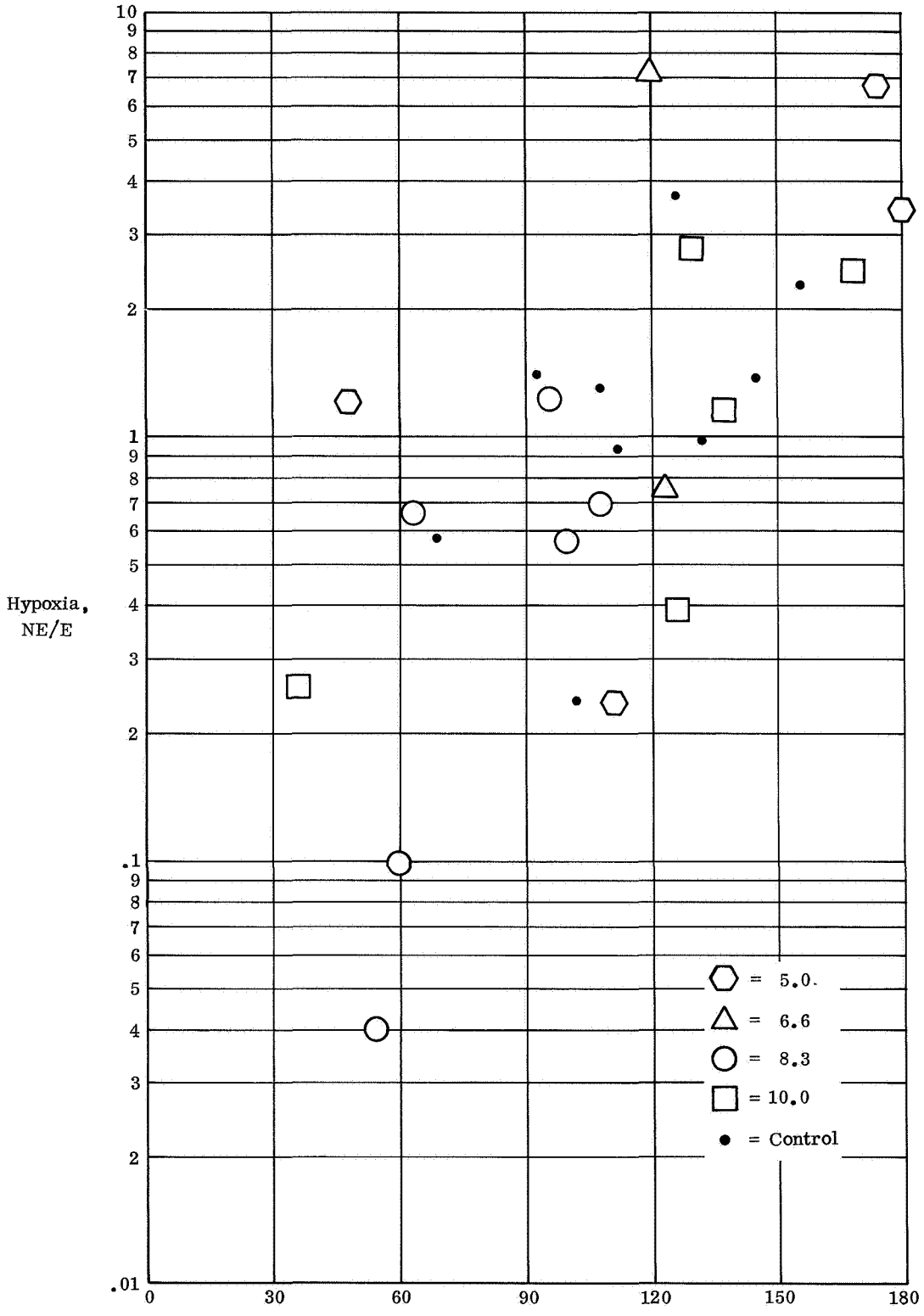
1630 CV 93 T 43

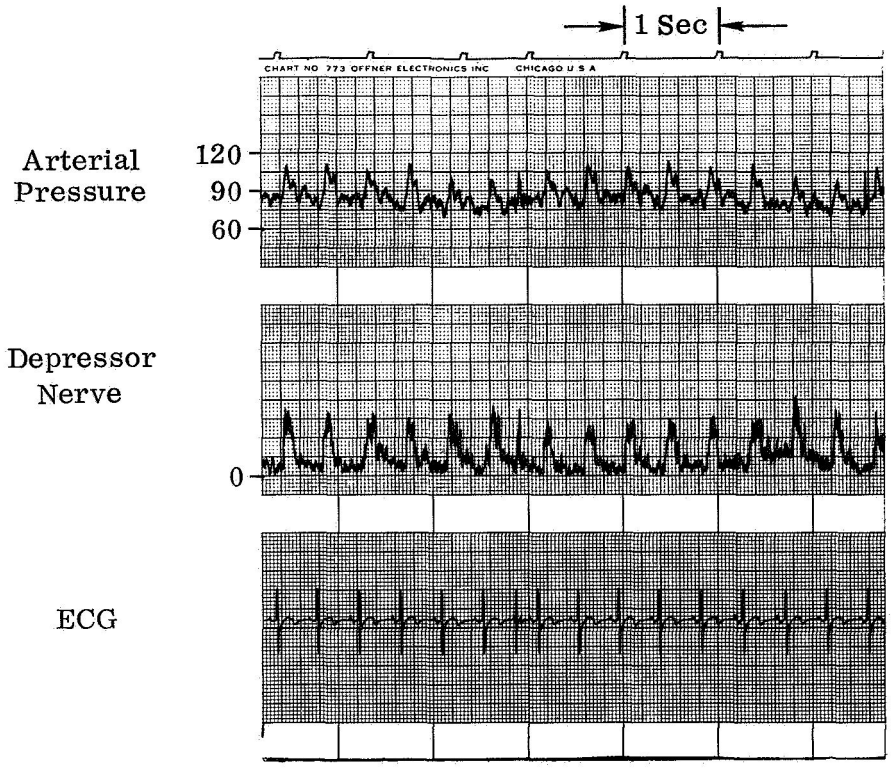
Correlograms: τ = 0.8 sec

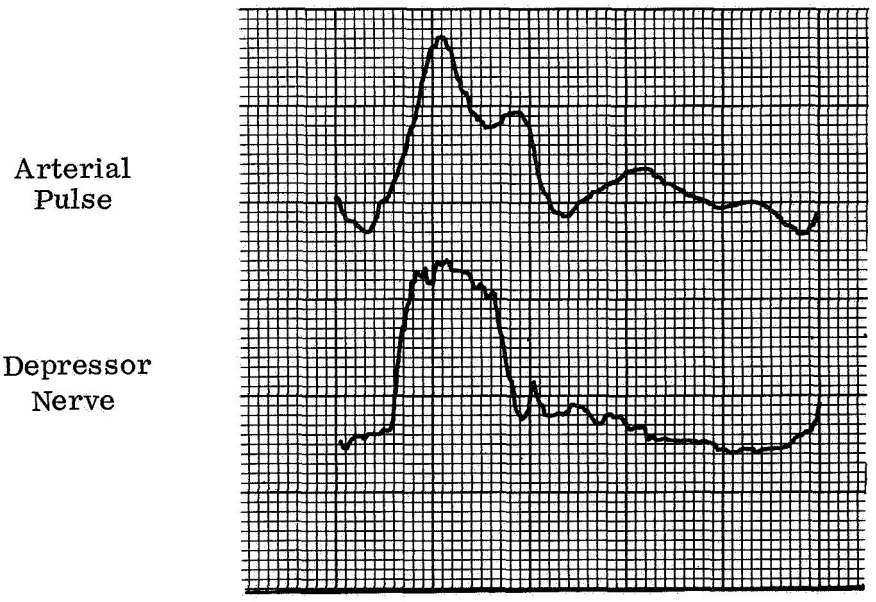

1 Min
Arterial Pressure, mmHg
180
120
60
0
Venous Pressure, cm H_2O
24
12
0
Resp.
Inflation
ECG
Mean Arterial Pressure
180
120
60
0
Adrenal Gland DC Potential
-1
0
+1

CV 166

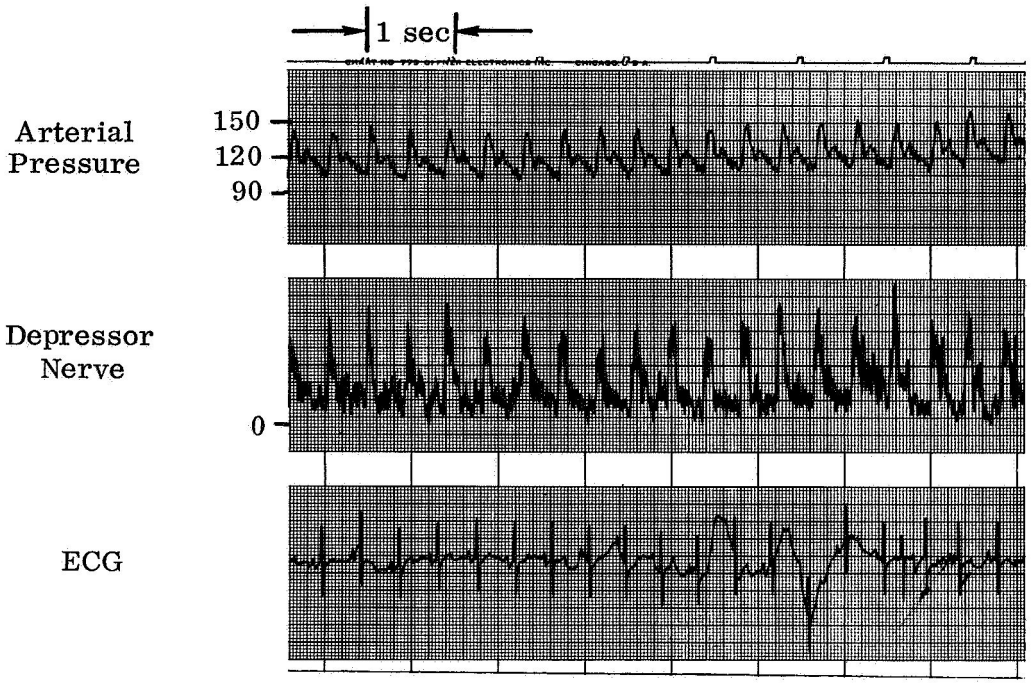

Hypoxia, E+NE, μg/min
10
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
.1
9
8
7
6
5
4
3
.01
30
60
90
120
150
180
Mean Arterial Pressure, mm Hg
= 5.0
= 6.6
= 8.3
= 10.0

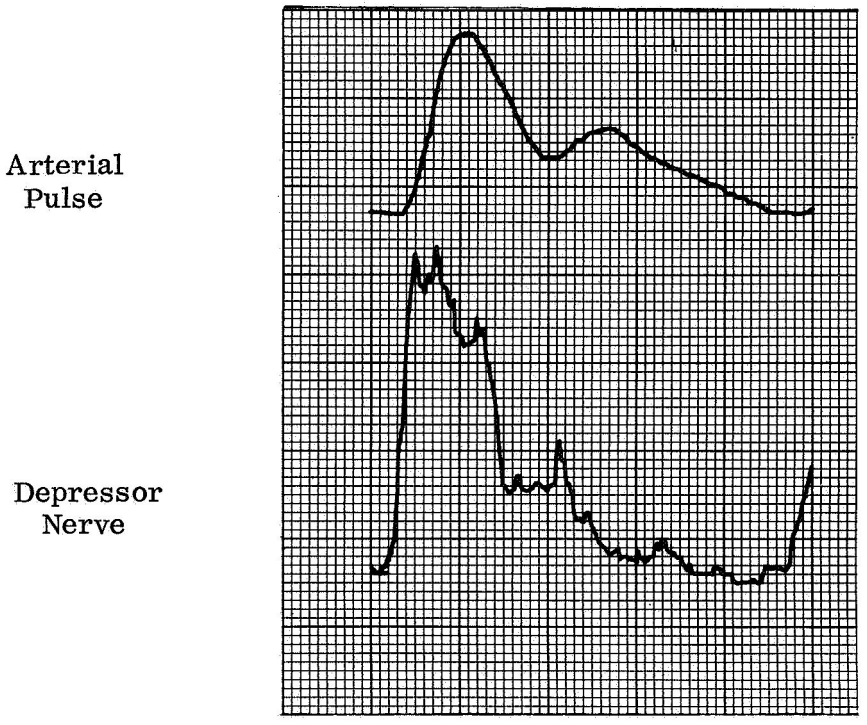

Hypoxia, NE/E
10
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
.1
9
8
7
6
5
4
3
2
.01
0
30
60
90
120
150
180
= 5.0
= 6.6
= 8.3
= 10.0
= Control



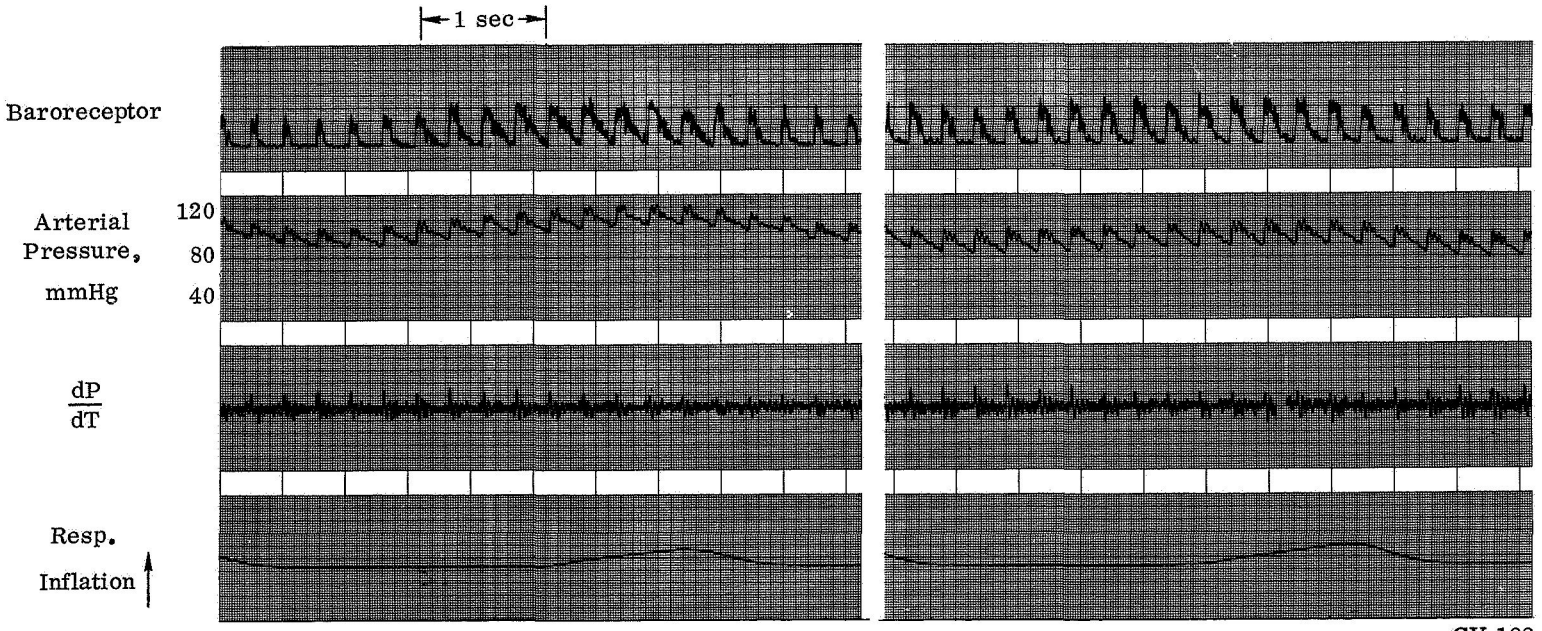


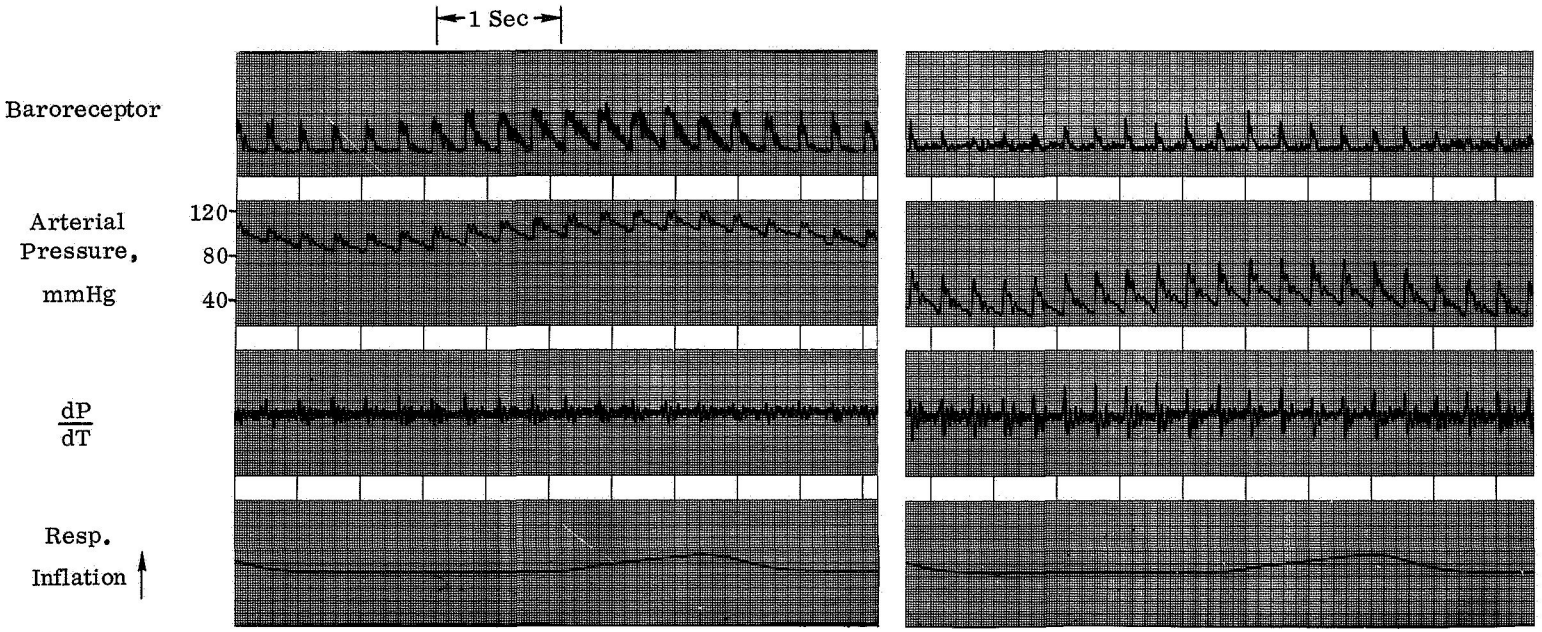
CV 164 T81-O 700-755

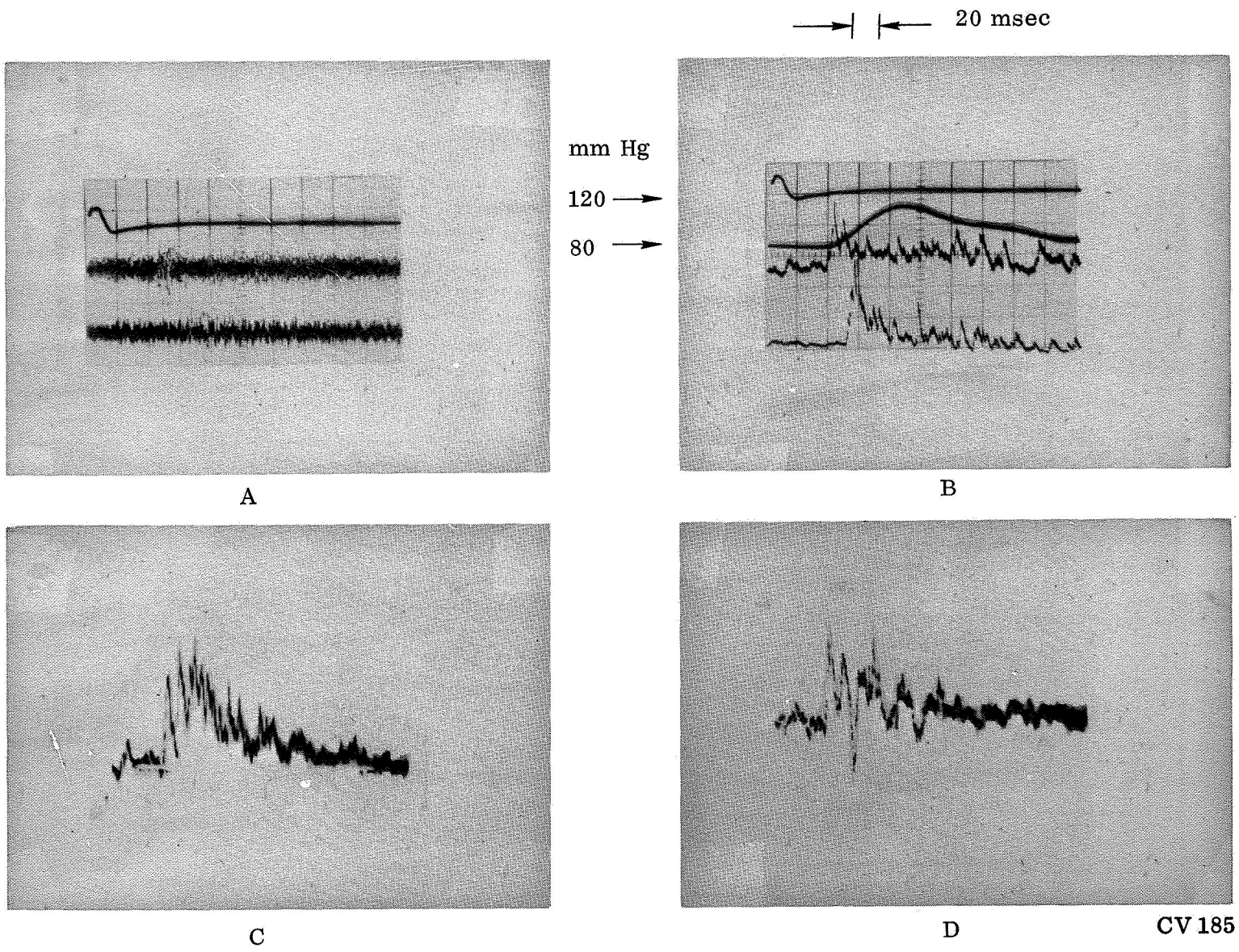




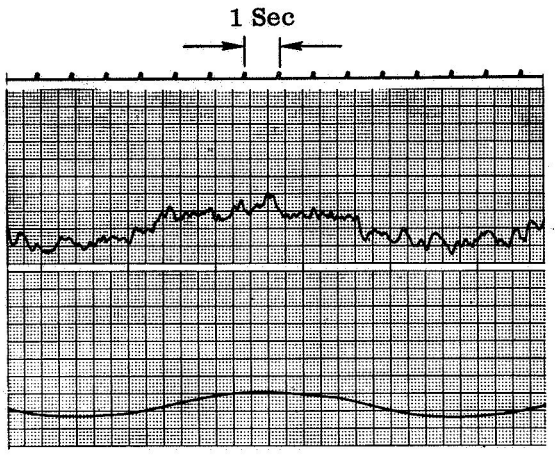
CV 164 T82-0 1950


1 sec
Baroreceptor
Arterial Pressure, mmHg
120
80
40
dP/dT
Resp.
Inflation
CV 182
Control
Arfonad & Aortic Clamping

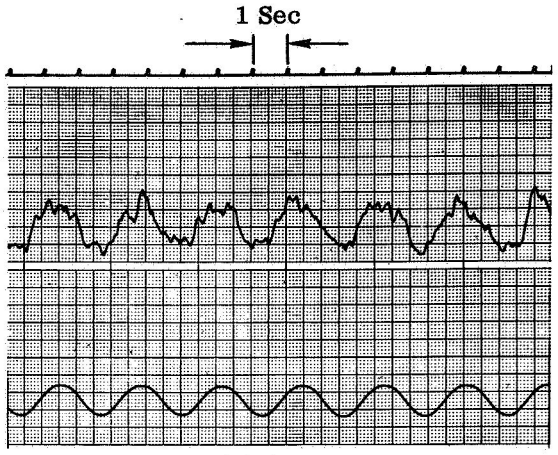

1 Sec
Baroreceptor
Arterial Pressure, mmHg
120
80
40
dP/dT
Resp.
Inflation
Control
Arfonad



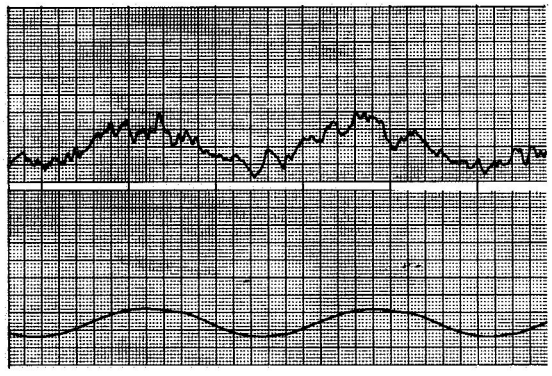
CV 185



5.5 cpm



27.3 cpm



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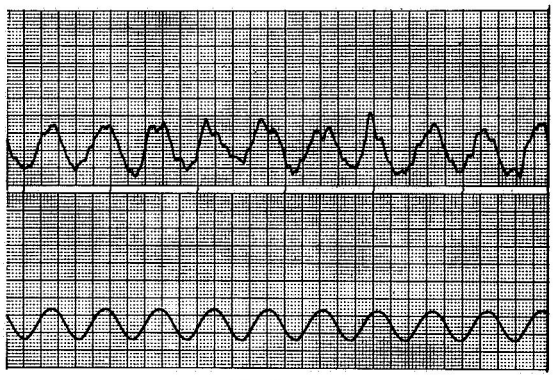
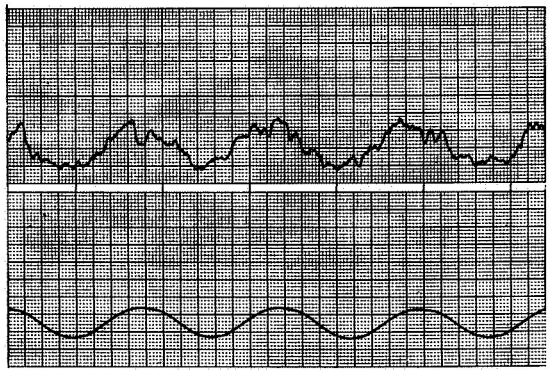


38.6 cpm



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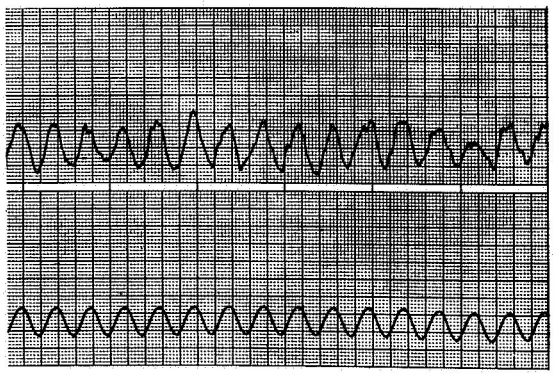

60.0 cpm

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12–6 Neural Monitoring of Pulmonary Ventilation

N. A. Normann
Bio-Sciences and Technology Department
Hamilton Standard Division
United Aircraft Corporation
Windsor Locks, Connecticut, U.S.A.

M. A. Bianchi
Bio-Sciences and Technology Department
Hamilton Standard Division
United Aircraft Corporation
Windsor Locks, Connecticut, U.S.A.

A. T. Gelman
Bio-Sciences and Technology Department
Hamilton Standard Division
United Aircraft Corporation
Windsor Locks, Connecticut, U.S.A.

The vagus nerve contains fibers which carry signals originating in pulmonary stretch receptors. It has been shown that the impulse frequency in the individual fibers increases with expansion of the lungs and the trachio-bronchial tree.[1,2] The purpose of the present study is to examine the feasibility of extracting this sensory information from the intact vagus nerve and to establish the relationship between the impulse patterns and pulmonary ventilation.

Methods. In anesthetized cats, the right vagus was exposed in the neck. Two pair of electrodes were placed on it. After amplification, the two channels of nerve signals were recorded on tape, together with the data from the following measurements: Pulmonary expansion was measured by utilizing a spirometer and by transducers sensing the movements of chest and abdominal walls. Multiplied by suitable coefficients, the latter quantities were electronically added to give the sum of wall movements. Air flow was measured with a hot-wire anemometer. Played back from the tape recorder, the nerve signals were analyzed by cross-correlation, and the resulting impulse pattern was displayed on a real time basis together with the transducer data.

Results. In most cats weighing less than 2½ kg, nerve traffic from pulmonary stretch receptors can be obtained from the intact vagus without splitting the epineurium. Results of cross-correlation analysis indicated maximum activity in fibers conducting impulses at speeds of 35 to 45 meters per second. A near linear relationship was found between the amplitudes of summed wall movements and of neural activity (Fig. 1). In the curarized animal, the linear relationship appeared to hold over a wide range of controlled amplitudes (Fig. 2).

Discussion and Conclusions. The conduction velocities of the fibers monitored belong to a group which is dominated by slowly adapting fibers.[3,4] In response to a sinusoidal inflation (curarized animal), 5 to 60 cycles per min., the waveform of the nerve traffic parallels that of the chest-abdominal walls, without phase shift and with little change in amplitude. The linear relationship between the combined axes ("radii") of the chest and the power density of the nerve impulses disagrees with Adrian's results, but is consistent with those of Widdicombe (an approximately linear proportionality between cube root of his volume values and impulse frequency). As an extension of this approach, electrodes have been implanted on the vagus, allowing monitoring in the intact, unanesthetized animal. In conclusion, it appears feasible to extract from the intact vagus information concerning pulmonary ventilation.

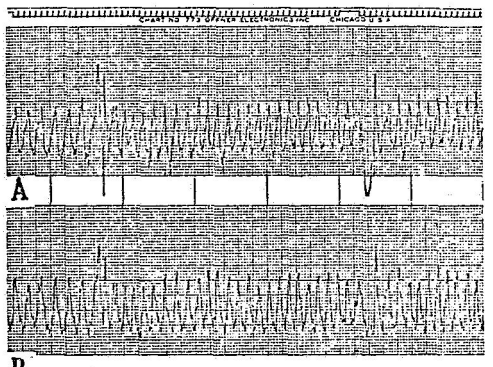

Fig. 1. Spontaneous breathing. A. Sum of two chest radii. B. Pattern in sensory nerve fibers. Time marker: min. and sec.

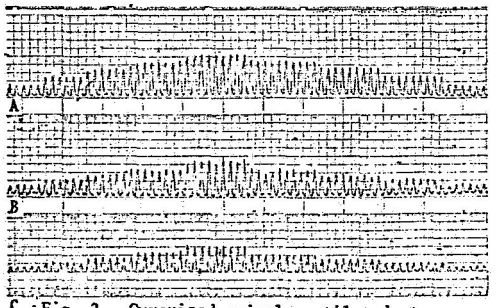

Fig. 2. Curarized animal ventilated at varying amplitudes of inflation. A. Sum of two chest radii. B. Pattern in sensory nerve fibers. C. Inflation pressure.

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